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TSCA HEALTH & SAFETY STUDY COVER SHEET • revised 6/25/96

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3.0 SUBMISSION TYPE Contains CBI
 8(d) 8(a) FYI 4 OTHER: Specify _____
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continuation sheet attached

2.1 SUMMARY/ABSTRACT ATTACHED
(may be required for 8(e); optional for §4, 8(d) & FYI)

2.2 SUBMITTER TRACKING
NUMBER OR INTERNAL ID

2.3 FOR EPA USE ONLY

YES NO

3.0 CHEMICAL/TEST SUBSTANCE IDENTITY Contains CBI

Reported Chemical Name (specify nomenclature if other than CAS name):

CAS# 344-04-7

Purity 99.9 %

Single Ingredient
 Commercial/Tech Grade
 Mixture

Bromopentafluorobenzene

COMPANY SANITIZED

Trade Name: BPFB Common Name: _____

CAS Number

NAME

% WEIGHT

Other chemical(s) present
in tested mixture

363-72-4

Pentafluorobenzene

0.1%

continuation sheet attached

4.0 REPORT/STUDY TITLE Contains CBI

Twenty-Eight-Day Repeated-dose Oral Toxicity Study of BPFB in Rats

continuation sheet attached

5.1 STUDY/TSCATS INDEXING TERMS
(CHECK ONE)

HEALTH EFFECTS (HE): HE ENVIRONMENTAL EFFECTS (EE): EE ENVIRONMENTAL FATE (EF): EF

5.2 STUDY/TSCATS INDEXING TERMS (see instructions for 4 digit codes)

STUDY SUBJECT ROUTE OF VEHICLE OF
TYPE: STOX ORGANISM (HE, EE only): RATS EXPOSURE (HE only): ORAL EXPOSURE (HE only)
Other: Other: Other: Other: olive oil

6.0 REPORT/STUDY INFORMATION Contains CBI Study is GLP

Laboratory _____ Report/Study
Date _____

Source of Data/Study Sponsor (if different than submitter) _____ Number of pages 4-111

continuation sheet attached

7.0 SUBMITTER INFORMATION Contains CBI

Submitter: _____ Title: _____ Phone: () _____

Company Name: _____ Company Address: _____

Technical Contact: _____ Submitter Address (if different): _____ Phone: () _____

continuation sheet attached

8.0 ADDITIONAL/OPTIONAL STUDY COMMENTS Contains CBI

Chromosomal Aberration Test of BPFB Using Cultured Mammalian Cells: In the test of chromosomal aberration using cultured mammalian cells (CHL cells) with S9 MIX by metabolic activation method, Bromopentafluorobenzene induced chromosomal aberration. D₂₀ values was 590 µg/ml.

continuation sheet attached

Submitter Signature: _____ Date: 9/10/97
Page 1 of 144

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	LIST OF ATTACHMENTS	Attachment page number(s)	Confidential
1	2.1 SUMMARY/ABSTRACT ATTATHED	3	X
2	6.0 REPORT/STUDY INFORMATION :Continuation sheet attached Final Report "Twenty-eight-day Repeated-dose oral Toxicity Study of BPFB in Rats"	4-111	X
3	8.0 ADDITIONAL/OPTIONAL STUDY COMMENTS :Continuation sheet attached Additinal Study and Regal Status of BPFB	112	X
4	8.0 ADDITIONAL/OPTIONAL STUDY COMMENTS :Continuation sheet attached Final Report "Chromosomal Aberration Test of BPFB Using Cultured Mammalian Cells"	113-140	X
5	Material Safety Data Sheet (MSDS)	141-144	X

2.1 SUMMARY/ABSTRACT ATTATHED

ABSTRACT

1 STUDY

Twenty-eight-day Repeated-dose oral Toxicity Study of BPFB in Rats

2 ABSTRACT

In the study of 28-day repeated dose oral toxicity, Bromopentafluorobenzene(BPFB) was shown that incisor of male and female rats in the 300 mg/kg/day were given degeneration and irregular alignment of ameloblasts at stage of maturation in the 14-day recovery test. Males in the 300 mg/kg/day were given a loss of incisor, swelling of the gingia and malocclusion in the same test.

The 28-day reoeated-dose caused pathalogogic change of liver and blood parameters of rats in 30-300 mg/kg/day. NOEL (no observed effects level) was 10mg/kg/day.

3 ABSTRACTOR

Receipt No. D95-1528
Report No. D-4847

STUDY CODE : B11-0394

FINAL REPORT

TWENTY-EIGHT-DAY REPEATED-DOSE
ORAL TOXICITY STUDY OF
BPFB
IN RATS

March, 1997

I, the undersigned, hereby declare that this report provides a correct English translation of the final report (Study Code B11-0394, issued on March 24, 1997).

July 23, 1997

Date

QUALITY ASSURANCE STATEMENT

Sponsor:

Title: Twenty-Eight-Day Repeated-Dose Oral Toxicity Study of BPFB in Rats
Study code: B11-0394

This report was audited by the Quality Assurance Section.
I, the undersigned, hereby declare that this report reflects
the original Japanese report.

Section Chief, Quality Assurance

GLP STATEMENTSponsor:Title: Twenty-Eight-Day Repeated-Dose Oral Toxicity Study of BPFB in RatsStudy Code: B11-0394

I, the undersigned, hereby declare that this study was conducted in compliance with "Concerning Testing Facilities Stipulated in Article 4 of the Order Prescribing the Items of the Test Related to the New Chemical Substances and of the Toxicity Investigations Related to the Designated Chemical Substances (Notification No. 39 of the Planning and Coordination Bureau, Environment Agency (EA), No. 229 of the Pharmaceutical Affairs Bureau, Ministry of Health and Welfare (MHW) & No. 85 (1984) of the Basic Industries Bureau, Ministry of International Trade and Industry (MITI), March 31, 1984; Notification No. 233 of the Planning and Coordination Bureau, EA, No. 38 of the Pharmaceutical Affairs Bureau, MHW & No. 823 (1988) of the Basic Industries Bureau, MITI, revised on November 18, 1988)" and with "Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice (May 12, 1981)".

Management: Signed in original March 24, 1997

QUALITY ASSURANCE STATEMENTSponsor:Title: Twenty-Eight-Day Repeated-Dose Oral Toxicity Study of BPFB in RatsStudy Code: B11-0394

This study was audited by Quality Assurance Section and the study procedures were inspected on the following dates.

Dates of Inspections and Audits	Dates of Reports to Study Director	Dates of Reports to Management
September 9, 1996	September 11, 1996	September 11, 1996
October 29, 1996	October 30, 1996	October 30, 1996
November 6, 1996	November 6, 1996	November 6, 1996
November 8, 1996	November 8, 1996	November 8, 1996
November 11, 1996	November 12, 1996	November 12, 1996
December 3, 1996	December 11, 1996	December 11, 1996
December 4, 1996	December 11, 1996	December 11, 1996
December 18, 1996	December 25, 1996	December 25, 1996
December 24, 1996	December 25, 1996	December 25, 1996
February 18, 1997	February 20, 1997	February 20, 1997
February 19, 1997	February 20, 1997	February 20, 1997
March 11, 1997	March 11, 1997	March 12, 1997
March 12, 1997	March 12, 1997	March 13, 1997
March 21, 1997	March 24, 1997	March 24, 1997
March 24, 1997	March 24, 1997	March 24, 1997

I, the undersigned, hereby declare that this report provides an accurate description of the methods and procedures used in this study and that the reported results accurately reflect the raw data obtained.

Section Chief, Quality Assurance:

Signed in original

March 24, 1997

Study Code: B11-0394
Test Substance Code: HR3322
Sponsor Code: N-080

TITLE

Twenty-Eight-Day Repeated-Dose Oral Toxicity Study of BPFB in Rats

SPONSOR

TESTING FACILITY

PURPOSE OF STUDY

The purpose of this study is to define the type, severity and reversibility of signs of the toxicity and to determine the no-observed-effect-level (NOEL) of the test substance by observing the functional and morphological changes in animals receiving repeated doses for 28 days.

TESTING METHOD

This study was conducted in accordance with "28-day Repeated Dose Toxicity Study in Mammalian Species" prescribed in "Notification on Partial Revision of Testing Methods Relating to the New Chemical Substances (Notification No. 700 of the Planning and Coordination Bureau, EA, No. 1039 of the Pharmaceutical Affairs Bureau, MHW & No. 1014 (1986) of the Basic Industries Bureau, MITI, December 5, 1986)"; and with "Section 407, Repeated Dose Oral Toxicity-Rodent: 28-day or 14-day Study" prescribed in the "OECD Guidelines for Testing of Chemicals (May 12, 1981)".

GLP COMPLIANCE

This study was conducted in conformity with "Concerning Testing Facilities Stipulated in Article 4 of the Order Prescribing the Items of the Test Related to the New Chemical Substances and of the Toxicity Investigations Related to the Designated Chemical Substances (Notification No. 39 of the Planning and Coordination Bureau, EA, No. 229 of the Pharmaceutical Affairs Bureau, MHW & No. 85 (1984) of the Basic Industries Bureau, MITI, March 31, 1984; Notification No. 233 of the Planning and Coordination Bureau, EA, No. 38 of the Pharmaceutical Affairs Bureau, MHW & No. 823 (1988) of the Basic Industries Bureau, MITI, revised on November 18, 1988)" and with "OECD Principles of Good Laboratory Practice (May 12, 1981)".

PERIOD OF STUDY

Commencement of Study:	September 9, 1996
Animal Receipt:	October 29, 1996
Start of Dosing:	November 5, 1996
Necropsy at the End of the Dosing Period:	December 3, 1996
Start of the Recovery Period:	December 3, 1996
Necropsy at the End of the Recovery Period:	December 17, 1996
Completion of Study:	March 24, 1997

LOCATION AND PERIOD FOR RETENTION OF RAW DATA AND SPECIMENS

Data and specimens, and each remaining lot of the test substance will be retained in the archives and test substance storage room, respectively, of for 10 years following the date of completion of study. After termination of the retention period, any measures taken will be done so with the approval of the sponsor. Samples and specimens that are liable to deteriorate markedly will be retained only for as long as the quality of the preparation permits evaluation with the sponsor's consent.

AUTHOR AND PERSONS CONCERNED WITH STUDY

Study Director: Signed in original March 24, 1997

Person in Charge of Pathologic Examination:

Signed in original March 21, 1997

Person in Charge of Clinical Chemistry:

Signed in original March 21, 1997

Study Staff:

Person in Charge of Divided Work:

Person in Charge of Chemical Analysis:

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APPENDIX **PHYSICOCHEMICAL REPORT**

SUMMARY

A 28-day repeated-dose oral toxicity study of BPFB followed by a 14-day recovery test was conducted in male and female Crj: CD (SD) rats (6/sex/ group), 5 weeks of age at the start of dosing. The highest dose was set at 300 mg/kg/day, and 3 lower doses at 100, 30 and 10 mg/kg/day. Recovery groups were separately provided for the 300, 100 mg/kg and the vehicle control groups.

No death occurred in accordance with test substance treatment. No abnormalities were noted in body weights and food consumption during the dosing period. In clinical signs, decreased spontaneous locomotion and salivation in the 300 mg/kg males and females, staining on the lower abdomen, staining around anus and moist hair of the lower abdomen in the 300 mg/kg females were noted during the dosing period. In hematological examinations, a decrease in platelet count was noted in the 300 mg/kg males at the end of the dosing period. In blood chemical examinations, increases in inorganic phosphorus levels in the 30, 100 and 300 mg/kg males, increases in total bilirubin levels in the 100 and 300 mg/kg females, increases in total cholesterol levels in the 300 mg/kg males and females, increases in GPT activity and alkaline phosphatase levels in the 300 mg/kg males, increases in GOT activity, triglyceride, inorganic phosphorus levels and a decrease in cholinesterase level in the 300 mg/kg females were noted at the end of the dosing period. In urinalysis, increases in urine volumes were noted in the 300 mg/kg males and females at the end of the dosing period. In organ weights, increases in liver weights in the 30, 100 and 300 mg/kg males and females, a decrease in spleen weight in the 300 mg/kg males and increases in kidney weights in the 300 mg/kg females were noted at the end of the dosing period.

In necropsy, enlargement of the liver were noted in the 300 mg/kg males and females at the end of the dosing period. In histopathological examinations ground glass appearance of the hepatocytes and swelling of the hepatocytes were noted in the 300 mg/kg males and females. Prominent nucleoli of the hepatocytes was noted in the 300 mg/kg males.

In the recovery test, an increase in GPT activity in the 300 mg/kg males and staining around the anus in the 300 mg/kg females were noted. Whitish region of the incisor in the 300 mg/kg males and females, loss of incisor, swelling of the gingiva and

malocclusion in the 300 mg/kg males were newly noted in clinical signs. In necropsy, whitish region of the incisor in the 300 mg/kg males and females, swelling of the gingiva in the 300 mg/kg males were noted. In histopathological examinations of the incisors, degeneration and irregular alignment of the ameloblasts at the stage of maturation were noted in the 300 mg/kg males and females.

In conclusion, NOEL of BPFB for rat was considered to be 10 mg/kg/day under the conditions tested.

MATERIALS AND METHODS

1. TEST SUBSTANCE (INFORMATION PROVIDED BY THE SPONSOR)

1.1 Name

Bromopentafluorobenzene

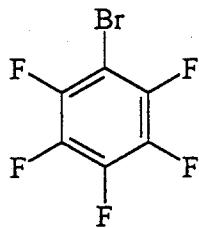
Other Name: BPFB

CAS No.: 344-04-7

1.2 Lot No.

1.3 Supplier

1.4 Structural Formula or Rational Formula (or Outline of Manufacturing Method, in Case Both are Unknown)

(Molecular Formula C₆BrF₅)

1.5 Purity

99.9 w/w%

1.6 Name and Concentration of Impurities

Pentafluorobenzene 0.1 w/w%

1.7 Physicochemical Properties

Appearance at Ordinary Temperature: Liquid

Molecular Weight: 246.96

Stability: The stability test was conducted in our laboratories, and confirmed that the test substance was stable during the dosing period.

Melting Point: -31°C

Boiling Point: 137°C

Vapor Pressure: —

Partition Coefficient: —

Solubility: Oil soluble

Degree of Solubility: Water: —

DMSO: —

Acetone: —

Others: —

Density

1.981 g/cm³

1.8 Storage Conditions

Stored in the dark and cold place.

1.9 Care on Handling

Gloves, a mask, a head cap and a lab coat were worn.

2. ANIMALS

Rats (SPF) of the Crj: CD (SD) strain were obtained from Charles River Japan, Inc. (Hino Breeding Center; 735, Shimokomatsuki, Hino-cho, Gamo-gun, Shiga 529-16, Japan). The animals were quarantined and acclimatized, and healthy animals with favorable weight gains were allocated to groups to ensure homogeneity of mean body weight using body weight-stratified randomization for the test. The animals were 5 weeks old, and weighed 121.6-147.6 g for males and 103.2-127.7 g for females at the start of dosing. The animals were identified by ear-tagging.

3. HOUSING CONDITIONS

The barrier-system animal room was maintained at a temperature of $23\pm2^{\circ}\text{C}$ and a relative humidity of $55\pm10\%$ with 10-15 air changes per hour and artificial light for 12 hours (between 7:00 and 19:00). The animals were housed individually in a hanging stainless steel cage with wire-mesh floor (165 W × 300 D × 150 H mm, Tokiwa Kagaku Kikai). The trays were changed twice a week, cages once a week and racks once four weeks. The racks and cages were identified by individual cards. The animals had free access to an MF pelleted diet (Oriental Yeast Co., Ltd.) and water (chlorinated) from the Hita City supply via sipper tubes from automatic waterer. The diet and housing materials were autoclaved at 121°C for 30 minutes prior to use. Analysis of contaminants in both the diet and drinking water confirmed that they would not affect the test system.

No environmental deviations which might affect the test results were noted during the quarantine, acclimation and study period.

4. GROUPING

The grouping was as follows:

Group	Dose	Volume	Concent-	No. of Animals (Animal No.)	
	(mg/kg/day)	(ml/kg)	ration (%)	Males	Females
Vehicle control	0	10	0	6 (1 - 6)	6 (49 - 54)
Vehicle control (recovery)	0	10	0	6 (7 - 12)	6 (55 - 60)
Low dose	10	10	0.1	6 (13 - 18)	6 (61 - 66)
Intermediate dose (1)	30	10	0.3	6 (19 - 24)	6 (67 - 72)
Intermediate dose (2)	100	10	1.0	6 (25 - 30)	6 (73 - 78)
Intermediate dose (2) (recovery)	100	10	1.0	6 (31 - 36)	6 (79 - 84)
High dose	300	10	3.0	6 (37 - 42)	6 (85 - 90)
High dose (recovery)	300	10	3.0	6 (43 - 48)	6 (91 - 96)

Reason for dosage selection:

A 14-day repeated-dose oral preliminary toxicity study was carried out at 3 doses of 50, 250 and 1,000 mg/kg. As a result, All males and one female in the 1,000 mg/kg were died. Abnormalities were noted at 50, 250 and 1,000 mg/kg in clinical signs, blood chemical examinations and organ weights, at 250 and 1,000 mg/kg in histopathological examinations and at 1,000 mg/kg group in hematological examinations and necropsy. In the main study the maximum dose was set at 300 mg/kg and 3 lower doses at 100, 30 and 10 mg/kg. Recovery groups were set at the 300 and 100 mg/kg and vehicle control groups.

5. PREPARATION OF THE TEST SUBSTANCE

5.1 Preparation

The test substance was accurately weighed and mixed with olive oil (Lot No. 004RHM, Fujimi Pharmaceutical Co., Ltd.) to make 3.0 w/v% solution. Three doses of 1.0, 0.3 and 0.1 w/v% were diluted from 3.0 w/v% solution. These were prepared once per week.

5.2 Stability test

The stability of the test substance in above preparations were confirmed by our laboratories.

6. DOSING

Treatment by oral gavage was carried out daily using a Nelaton catheter (Terumo Corporation) and a syringe (Terumo Corporation) in the morning for 28 days, and the subsequent 14 days were used as a recovery period.

7. OBSERVATION AND MEASUREMENT

The day of the start of dosing was defined as day 1, and the day before as day -1. The week of the start of dosing period was defined as week 1. Also, the next day of the final dosing was defined as recovery day 1, and the week of the start of the recovery period as recovery week 1.

7.1 Clinical Signs

All animals were observed at least once per day.

7.2 Body Weight

All animals were weighed as follows:

Before Dosing: day -2 (at the time of grouping)

During the Dosing Period: day 1 (at the start of dosing), 3, 5, 8, 10, 12, 15,
17, 19, 22, 24, 26 and 28

During the Recovery Period: day 1 (at the start of the recovery period), 3, 5, 8,
10, 12 and 14

In addition, immediately before necropsy, body weights was measured for calculation of relative organ weights.

7.3 Food Consumption

Food consumption was measured as follows:

Before Dosing: Once

During the Dosing and Recovery Periods: Twice a week

7.4 Hematological Examinations

All survival were fasted overnight (16-20 hours) at the end of the dosing and recovery periods, and blood samples were taken from abdominal aorta under ether anesthesia. Sodium citrate was used for examinations of prothrombin time and activated partial thromboplastin time, and EDTA-2K was used for another parameters as an anticoagulant. The following parameters were examined for the blood and plasma samples obtained.

Parameter		Method
1) Red Blood Cell Count (RBC)	($\times 10^4/\text{mm}^3$)	System for detecting change in electrical resistance
2) White Blood Cell Count (WBC)	($\times 10^2/\text{mm}^3$)	System for detecting change in electrical resistance
3) Hemoglobin Conc. (Hb)	(g/dl)	Oxyhemoglobin method
4) Hematocrit Value (Ht)	(%)	System for detecting pulse
5) Mean Corpuscular Volume (MCV)	(μm^3)	$\frac{\text{Ht}}{\text{RBC}} \times 10^3$
6) Mean Corpuscular Hemoglobin (MCH)	(pg)	$\frac{\text{Hb}}{\text{RBC}} \times 10^3$
7) Mean Corpuscular Hemoglobin Conc. (MCHC) (%)	(%)	$\frac{\text{Hb}}{\text{Ht}} \times 10^2$
8) Platelet Count	($\times 10^4/\text{mm}^3$)	System for detecting change in electrical resistance
9) Reticulocytes Count	(%)	New methylene blue staining
10) Prothrombin Time (PT)	(sec)	Magnetic sensor system
11) Activated Partial Thromboplastin Time (APTT)(sec)		Magnetic sensor system
12) Differentiation of Leukocytes	(%)	Wright staining
Stab Neutrophils (N-Band)		
Segmented Neutrophils (N-Seg)		
Eosinophils (Eosino)		
Basophils (Baso)		
Lymphocytes (Lymph)		
Monocytes (Mono)		

1) - 8)	Microcell counter M-2000, Toa Medical Electronics
9), 12)	MICROX HEG-120A, OMRON
10), 11)	KC-10A, Amelung

7.5 Blood Chemical Examinations

Sera were separated from the blood samples collected at the same time of the hematological examinations, and examined as follows.

Parameter		Method
1) GOT	(IU/l)	UV method (Method based on JSCC)
2) GPT	(IU/l)	UV method (Method based on JSCC)
3) Alkaline Phosphatase (ALP)	(IU/l)	p-Nitrophenyl phosphate method
4) Cholinesterase (ChE)	(IU/l)	Butyrylthiocholine iodide method
5) γ -GTP	(IU/l)	L- γ -Glutamyl-p-nitroanilide method
6) Total Cholesterol (T-Chol)	(mg/dl)	COD-DAOS method
7) Triglyceride (TG)	(mg/dl)	GPD-DAOS method
8) Glucose	(mg/dl)	Glucokinase-G-6-PDH method
9) Total Protein (T-Protein)	(g/dl)	Biuret method
10) Albumin	(g/dl)	Bromocresol green method
11) A/G Ratio		Albumin T-Protein - Albumin
12) Blood Urea Nitrogen (BUN)	(mg/dl)	Urease indophenol method
13) Creatinine	(mg/dl)	Jaffé's method
14) Total Bilirubin (T-Bil)	(mg/dl)	Azo bilirubin method
15) Calcium (Ca)	(mg/dl)	OCPC method
16) Inorganic Phosphorus (IP)	(mg/dl)	Fiske-Subbarow method
17) Sodium (Na)	(mEq/l)	Crown-Ether membrane electrode method
18) Potassium (K)	(mEq/l)	Crown-Ether membrane electrode method
19) Chloride (Cl)	(mEq/l)	Coulometric titration method

1) - 10), 12) - 16) 7150 Automatic Analyzer, Hitachi

17) - 19) PVA- α III, A & T

7.6 Urinalysis

Sixteen-hour urine samples were collected in individual metabolic cages, from all animals at day 28 and recovery day 14 and examined for volume, color and additional items of pH, protein, ketone bodies, bilirubin, occult blood, glucose and urobilinogen using a test paper (N-Multistix®, Bayer-Sankyo).

7.7 Necropsy

All animals were necropsied in detail to record.

7.8 Organ Weights

The following organs were weighed wet in all animals:

Brain, liver, kidneys, spleen, adrenal glands, testes (or ovaries)

7.9 Histopathological Examinations

- 1) The following organs and tissues from all animals were preserved in 10% formalin:

Brain (cerebrum, cerebellum), hypophysis, eyeball, thyroid glands (with parathyroid glands), heart, lung, liver, kidneys, spleen, adrenal glands, stomach, intestine (duodenum to rectum), testes (or ovaries), urinary bladder, bone marrow (femur), incisors (at the end of the recovery period), gross lesions

- 2) Light microscopic examinations were performed on the following organs and tissues except incisors, after paraffin embedding and sectioning followed by hematoxylin and eosin staining at Bio pathology Institute, Ltd. (1200-2, Ohara, Kunisaki-machi, Higashikunisaki, Oita 873-05, Japan).

At the End of the Dosing Period

Vehicle control and 300 mg/kg groups: Liver, kidneys, spleen, heart, stomach, intestine (duodenum, jejunum, ileum, cecum, colon, rectum), adrenal glands

100 mg/kg group: Liver

30 mg/kg groups: Liver (males)

At the End of the Recovery Period

Vehicle control and 300 mg/kg groups: Liver, incisors

100 mg/kg group

Liver (males), incisors

Gross Lesions

At the End of the Dosing Period

Female, 10 mg/kg group (No. 61): Glandular stomach

At the End of the Recovery Period

Male, 300 mg/kg group (No. 44): Glandular stomach

100 mg/kg group (No. 35): Testes

Berlin blue staining was performed in incisors of all animals at the end of recovery period.

8. STATISTICAL ANALYSIS

Data regarding body weights, food consumption, hematological examinations, blood chemical examinations, urine volume and organ weights were analyzed using Bartlett's test for homogeneity of variance. If the variances were homogeneous at a significance level of 5%, one way analysis of variance was performed. When there was a significant difference in this analysis, the difference between the vehicle control group and each of the treatment group was analyzed by Dunnett's test.

If the variances were not homogeneous in the Bartlett's test, Kruskal-Wallis's test was used. When there was a significant difference in this test, the difference between the vehicle control group and each of the treatment group was analyzed by nonparametric Dunnett's.

UNEXPECTED SITUATIONS AND DEVIATIONS FROM PROTOCOL

One male (No. 45) of the 300 mg/kg group lost the right upper incisor during the recovery period and had difficulty in having perreted feed. Powdered feed was provided to this animal, and food consumption was not recorded for this animal during the period which thought decreased food consumption was occurred. The data of remaining 5 animals in this group were used for evaluation of the study results.

There were no other unexpected situations which might have affected the test results and deviations from protocol.

RESULTS

1. CLINICAL SIGNS (TABLE 1, ADDENDUM 1)

1.1 During the Dosing Period

Male: Decreased spontaneous locomotion (10/12) and salivation (12/12) were noted in the 300 mg/kg group. Salivation was noted in the vehicle control group (9/12), 10 mg/kg group (4/6), 30 mg/kg group (6/6) and 100 mg/kg group (11/12). Exudate (1/12), loss of hair (1/12) and scab formation (1/12) were noted in the 100 mg/kg group.

<Condition of salivation>

Vehicle control group: sporadically after dosing from day 4 or day 16.
10 mg/kg group: sporadically after dosing from day 4 or day 5.
30 mg/kg group: sporadically after dosing from day 4 or day 14.
100 mg/kg group: sporadically or continuously after dosing from day 4 or day 7.
300 mg/kg group: continuously before dosing, after dosing or afternoon from day 1 or day 6.

Female: Decreased spontaneous locomotion (5/12), salivation (12/12), staining on the lower abdomen (1/12), staining around the anus (1/12) and moist hair of the lower abdomen (1/12) were noted in the 300 mg/kg group. Salivation was noted in the vehicle control group (5/12), 10 mg/kg group (3/6), 30 mg/kg group (6/6) and 100 mg/kg group (11/12).

<Condition of salivation>

Vehicle control group: sporadically after dosing from day 7 or day 24.
10 mg/kg group: sporadically after dosing from day 8.
30 mg/kg group: sporadically after dosing from day 4 or day 15.
100 mg/kg group: sporadically or continuously after dosing from day 4 or day 15.
300 mg/kg group: continuously after dosing or afternoon from day 3 or day 5.

1.2 During the Recovery Period

Male: Whitish region of the incisors (6/6), loss of the right upper incisor (1/6), swelling of the gingiva (1/6) and malocclusion (1/6) were noted in the 300 mg/kg group. Loss of hair (1/6) and scab formation (1/6) were noted in the 100 mg/kg group.

Female: Whitish region of the incisors (6/6) and staining around the anus (1/6) were noted in the 300 mg/kg group.

2. BODY WEIGHTS (FIG.1, TABLE 2, ADDENDUM 2)
 - 2.1 During the Dosing Period
No abnormalities were noted in both sexes.
 - 2.2 During the Recovery Period
No abnormalities were noted in both sexes.
3. FOOD CONSUMPTION (FIG.2, TABLE 3, ADDENDUM 3)
 - 3.1 During the Dosing Period
No abnormalities were noted in both sexes.
 - 3.2 During the Recovery Period
No abnormalities were noted in both sexes.
4. HEMATOLOGICAL EXAMINATIONS (TABLE 4, ADDENDUM 4)
 - 4.1 At the End of the Dosing Period
Male: A decrease in platelet count was noted in the 300 mg/kg group.
Decreases in hemoglobin conc. and hematocrit value were noted in the 10 mg/kg group.
Female: An increase in mean corpuscular hemoglobin was noted in the 300 mg/kg group.
 - 4.2 At the End of the Recovery Period
No abnormalities were noted in both sexes.
5. BLOOD CHEMICAL EXAMINATIONS (TABLE 5, ADDENDUM 5)
 - 5.1 At the End of the Dosing Period
Male: An increase in inorganic phosphate level was noted in the 30 mg/kg group.
An increase in inorganic phosphate level was noted in the 100 mg/kg group. Increases in GPT activity, alkaline phosphatase, total cholesterol and inorganic phosphate levels were noted in the 300 mg/kg group.
Female: An increase in total bilirubin was noted in the 100 mg/kg group.
Increases in GOT activity, total cholesterol, triglyceride, total bilirubin, inorganic phosphate levels and a decrease in cholinesterase activity were noted in the 300 mg/kg group.
 - 5.2 At the End of the Recovery Period
Male: An increase in GPT activity was noted in the 300 mg/kg group.
Female: A decrease in albumin level was noted in the 100 mg/kg group.

6. URINALYSIS (TABLE 6, ADDENDUM 6)

6.1 At the End of the Dosing Period

Male: An increase in urine volume was noted in the 300 mg/kg group.

Female: An increase in urine volume was noted in the 300 mg/kg group.

6.2 At the End of the Recovery Period

No abnormalities were noted in both sexes.

7. ORGAN WEIGHTS (TABLES 7,8, ADDENDA 7,8)

7.1 At the End of the Dosing Period

Male: An increase in relative liver weight was noted in the 30 mg/kg group. Increases in absolute and relative liver weights were noted in the 100 mg/kg group. Decreases in absolute and relative spleen weights and increase in liver weight were noted in the 300 mg/kg group. Increases in absolute brain and testis weights were noted in the 10 mg/kg group. An increase in absolute testis weights was noted in the 30 mg/kg group.

Female: An increase in relative liver weight was noted in the 30 mg/kg group. An increase in relative liver weight was noted in the 100 mg/kg group. Increases in absolute and relative liver and kidney weights were noted in the 300 mg/kg group. An increase in absolute spleen and liver weights were noted in the 10 mg/kg group. An increase in relative kidney weights were noted in the 30 mg/kg group.

7.2 At the End of the Recovery Period

No abnormalities were noted in both sexes.

8. NECROPSY (TABLE 9, ADDENDUM 9)

8.1 At the End of the Dosing Period

Male: Enlargement of the liver (5/6) and blackish region of the mucosa in the glandular stomach (1/6) were noted in the 300 mg/kg group.

Female: Enlargement of the liver (4/6) was noted in the 300 mg/kg group. Blackish region of the mucosa in the glandular stomach (1/6) was noted in the 10 mg/kg group. Blackish region of the mucosa in the glandular stomach (1/6) was noted in the 300 mg/kg group.

8.2 At the End of the Recovery Period

- Male: Swelling of the gingiva (1/6) and whitish region of the incisor (6/6) in the oral cavity were noted in the 300 mg/kg group. Small testis (1/6) in the 100 mg/kg group and blackish region of the mucosa in the glandular stomach (1/6) was noted in the 300 mg/kg group.
- Female: Whitish region of the incisor in the oral cavity (6/6) was noted in the 300 mg/kg group.

9. HISTOPATHOLOGICAL EXAMINATIONS (TABLE 10, ADDENDUM 9)**9.1 At the End of the Dosing Period**

- Male: Swelling of the hepatocytes in the liver (+, 2/6) was noted in the 100 mg/kg group. Ground glass appearance of the hepatocytes (+, 4/6; ++, 2/6), prominent nucleoli of the hepatocytes (+, 6/6) and swelling of the hepatocytes (++, 6/6) were noted in the 300 mg/kg group. Increased eosinophilic bodies in the kidney (++, 1/6) in the vehicle control group, increased eosinophilic bodies in the kidney (\pm , 2/6; +, 1/6) and necrosis of the mucosa in the glandular stomach (+, 1/6) in the 300 mg/kg group were noted.
- Female: Ground glass appearance of the hepatocytes (+, 4/6) and swelling of the hepatocytes (+, 6/6) were noted in the 300 mg/kg group. Mineralization in the corticomedullary junction in the kidney (+, 1/6) in the vehicle control group, necrosis of the mucosa in the glandular stomach (+, 1/1) in the 10 mg/kg group and necrosis of the mucosa in the glandular stomach (+, 1/6) in the 300 mg/kg group were noted.

9.2 At the End of the Recovery Period

- Male: Degeneration and irregular alignment of the ameloblasts at the stage of maturation (+, 6/6) were noted in the 300 mg/kg group. Atrophy of the seminiferous tubules (+++, 1/1) and interstitial cell hyperplasia (+, 1/1) in the 100 mg/kg group, microvesicular steatosis of the hepatocytes in the liver (+, 1/6) and necrosis of the mucosa in the glandular stomach (+, 1/1) were noted in the 300 mg/kg group.
- Female: Degeneration and irregular alignment of the ameloblasts at the stage of maturation (\pm , 3/6; +, 3/6) were noted in the 300 mg/kg group.

DISCUSSION

The oral toxicity of BPFB was examined in the SD strain male and female rats given a daily dose of 10, 30, 100 and 300 mg/kg/day for 28 days, followed by the 14-day recovery period.

No death occurred in accordance with test substance treatment.

No abnormalities were noted in body weights and food consumption during the dosing period.

In clinical signs, decreased spontaneous locomotion in the 300 mg/kg males and females, staining on the lower abdomen, staining around the anus and moist hair of the lower abdomen in the 300 mg/kg females were noted. Salivation was noted in all groups including the vehicle control. The salivation in the 300 mg/kg group was considered to be treatment related since this was not observed only after dosing, in lower doses the salivation was transient and there was no other change related to this, and it was not considered related to test substance.

In hematological examinations, a decrease in platelet count was noted in the 300 mg/kg males at the end of the dosing period. Although an increase in mean corpuscular hemoglobin was noted in the 300 mg/kg females, no abnormalities were noted in red blood cell count and hemoglobin conc., and the elevation was not considered to be test substance treatment.

In blood chemical examinations, increases in inorganic phosphate levels in the 30, 100 and 300 mg/kg males, increases in total bilirubine levels in the 100 and 300 mg/kg females were noted at the end of the dosing period. Increases in total cholesterol levels in the 300 mg/kg males and females, increases in GPT activity, alkaline phosphatase level in the 300 mg/kg males, increases in GOT activity, triglyceride and inorganic phosphate levels and a decrease in cholinesterase activity in the 300 mg/kg females were noted at the end of the dosing period.

In urinalysis, increases in urine volumes were noted in the 300 mg/kg males and females at the end of the dosing period.

In organ weights, increases in absolute liver weights in the 100 and 300 mg/kg males, a decrease in absolute spleen weight in the 300 mg/kg males and increases in absolute liver and kidney weights in the 300 mg/kg females were noted at the end of the dosing period. Increases in relative liver weights in the 30, 100 and 300 mg/kg males and females, a decrease in relative spleen weight in the 300 mg/kg males and an increase in relative kidney weights in the 300 mg/kg females were noted at the end of the dosing period.

In necropsy, enlargement of the liver was noted in the 300 mg/kg males and females. In these groups, and blackish region of the mucosa in the glandular stomach and necrosis of the mucosa equivalent for this were noted; however, these changes were not considered to be administration related since they were frequently observed in the vehicle control group and there was no other change related to them.

In histopathological examinations, swelling of the hepatocytes in the 100 mg/kg males, ground glass appearance of the hepatocytes, swelling of the hepatocytes in the 300 mg/kg males and females and prominent nucleoli of the hepatocytes in the 300 mg/kg males were noted.

In the recovery test, an increase in GPT activity in the 300 mg/kg males and staining around the anus in the 300 mg/kg females persisted to the recovery period necropsy. Whitish region of the incisors in the 300 mg/kg males and females, loss of the right upper incisor, swelling of the gingiva and malocclusion in the 300 mg/kg males were newly noted. In necropsy, whitish region of the incisor in the 300 mg/kg males and females, swelling of the gingiva in the 300 mg/kg males were noted. Degeneration and irregular alignment of the ameloblasts at the stage of maturation in the 300 mg/kg males and females were noted in histopathological examinations. At the end of the dosing period incisors were not examined in histopathological examinations since they were not collected. The other changes were not considered related to treatment, since there were no changes related to them and no abnormalities were noted during and at the end of the dosing periods.

The other changes and statistically significant differences were not considered related to administration of the test substance, since there was no dose-relationship and also noted in the vehicle control group.

The main effect of BPFB was observed in the liver and incisors, the lesion of incisor was only observed in the recovery test. This was considered that the change of the ameloblasts during the dosing period appeared later on, namely, the change occurred in the cells before the stage of maturation, then they entered the stage and the change of incisor was observed during the recovery period. At the end of the recovery period no abnormalities were noted in the ameloblasts before the stage of maturation, and it was considered that there were no effects of the test substance for the ameloblasts after dosing.

In conclusion, the NOEL of BPFB for rats in this study was considered to be 10 mg/kg/day based upon increases in relative liver weights in the 30, 100 and 300 mg/kg males and females and increases in inorganic phosphate levels in the 30, 100 and 300 mg/kg males.

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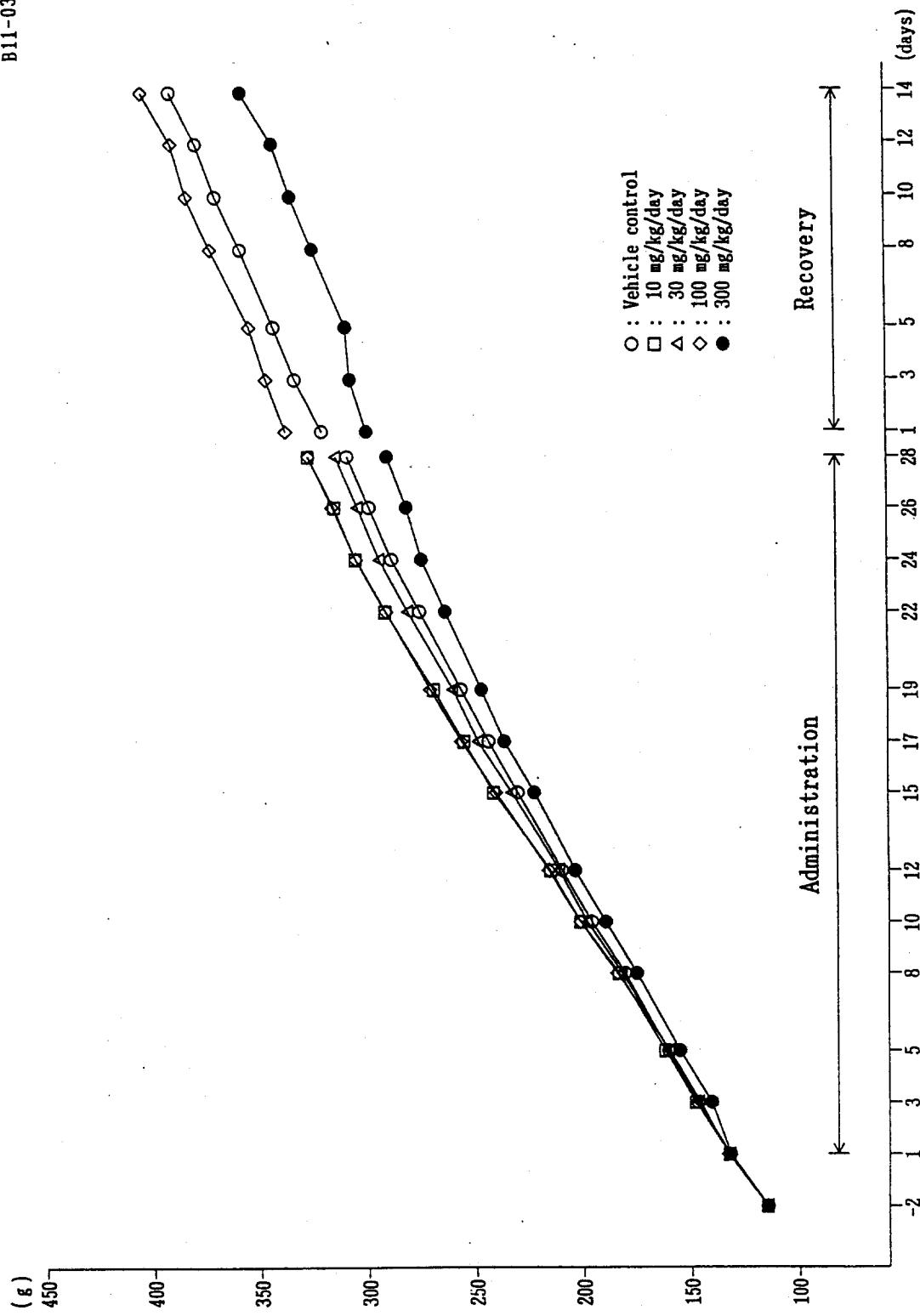


Figure 1 28-day repeated-dose oral toxicity study in rats
Mean body weights : Male

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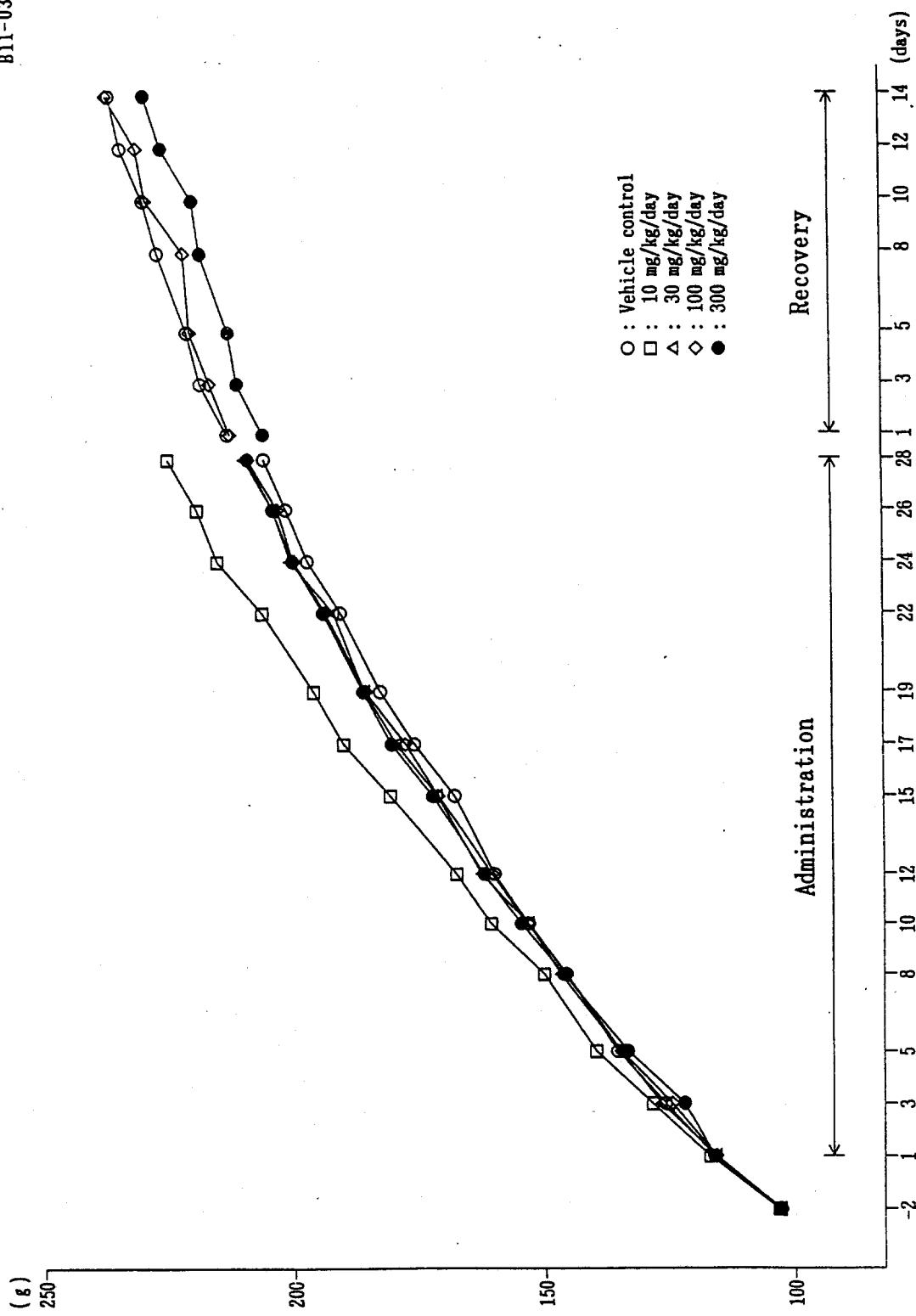


Figure 1 -Continued
Mean body weights : Female

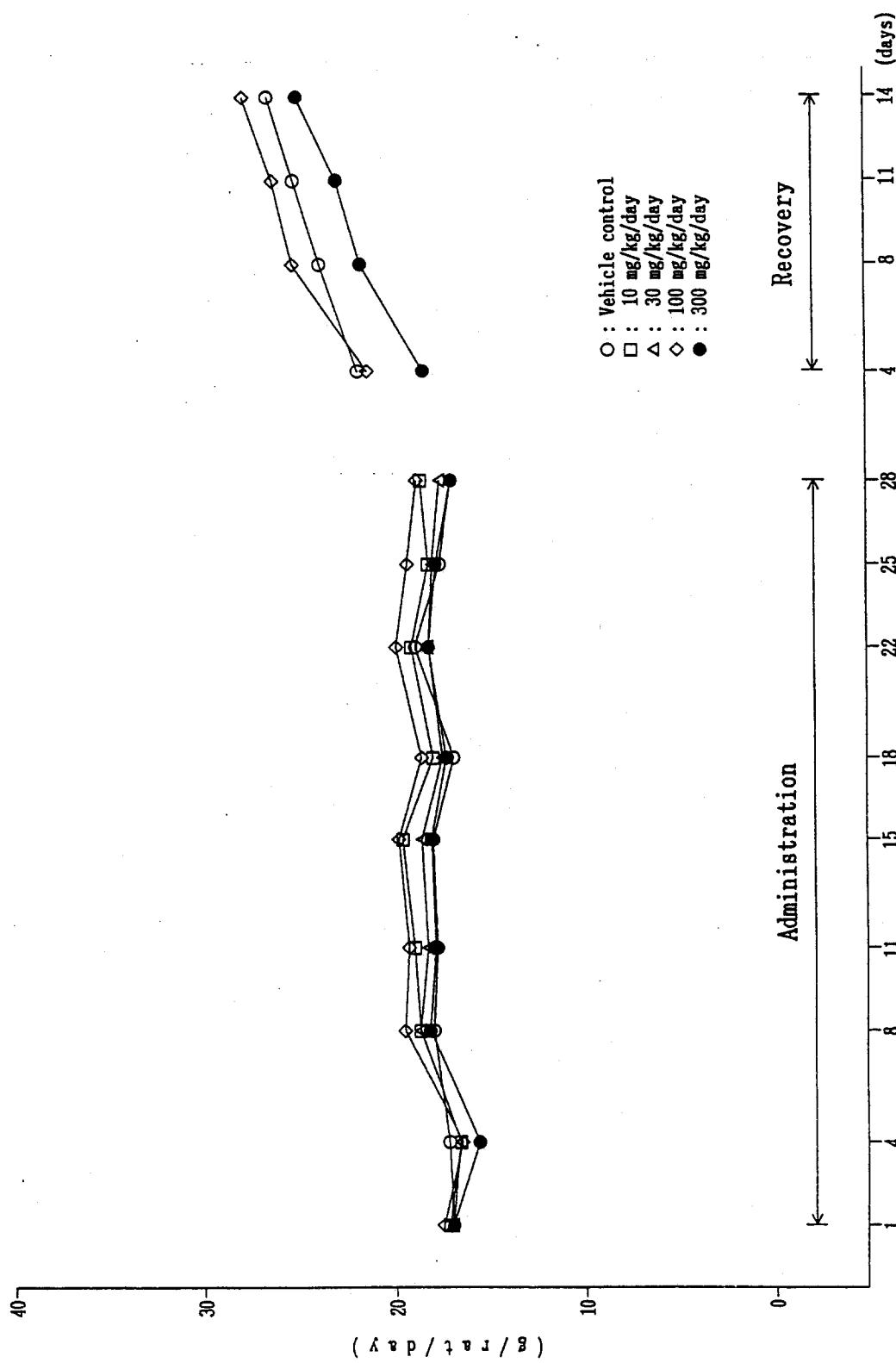


Figure 2 28-day repeated-dose oral toxicity study in rats
Mean food consumption : Male

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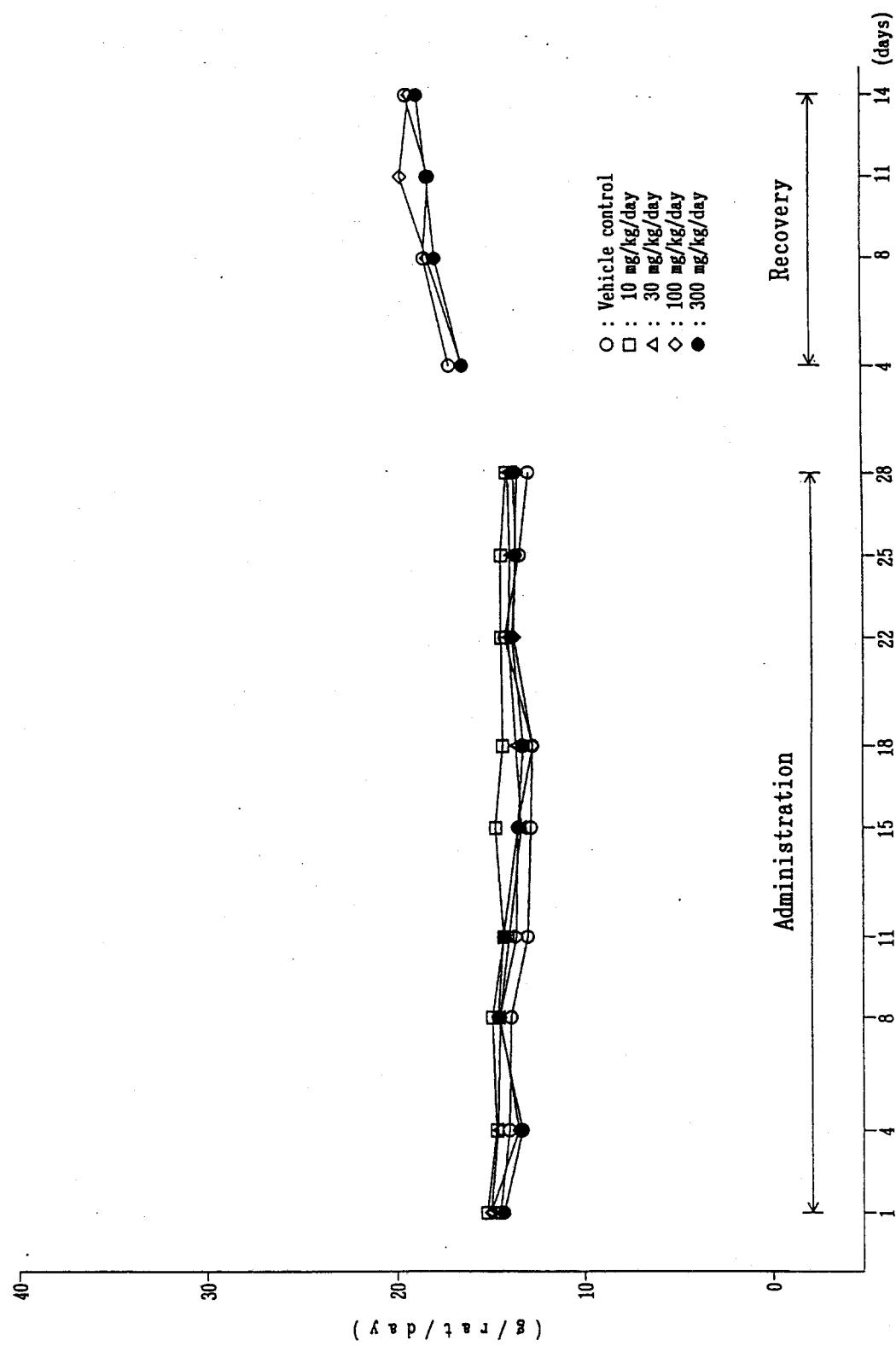


Figure 2 -Continued
Mean food consumption : Female

Table 1 28-day repeated-dose oral toxicity study in rats
Clinical signs

Sex	Signs	Number of animals (6 animals / group)											
		Administration period						Recovery period					
		Vehicle control	Vehicle control	10	30	100	300	300	300	300	300	(Recovery)	(Recovery)
	No abnormalities detected	2	1	2	1	1	1	1	1	1	1	6	5
	Salivation	4	5	4	6	5	6	6	6	6	6	6	6
	Decreased spontaneous locomotion												
	British region of incisor												
	Loss of incisor												
	Swelling of gingiva												
	Malocclusion												
	Exudate												
	Loss of hair												
	Scab formation												
	No abnormalities detected	4	3	3	3	3	3	3	3	3	3	6	6
	Salivation	2	3	3	3	3	6	6	6	6	6	6	6
	Decreased spontaneous locomotion												
	British region of incisor												
	Staining lower abdomen												
	Staining around anus												
	Woist hair of lower abdomen												

Table 2 28-day repeated-dose oral toxicity study in rats
Mean body weights(g)

Sex	Exp. group (mg/kg/day)	Number of animals	Administration					
			-2	1	3	5	8	10
Male	Vehicle control	12	114.5 ± 4.5	132.6 ± 7.0	147.0 ± 8.2	160.8 ± 10.7	180.8 ± 12.2	196.0 ± 15.3
	10	6	114.7 ± 4.9	132.6 ± 6.2	148.0 ± 8.5	162.1 ± 9.6	183.7 ± 11.7	201.2 ± 13.8
	30	6	114.6 ± 5.7	132.1 ± 6.2	146.6 ± 6.6	160.3 ± 7.2	182.2 ± 9.5	198.1 ± 10.8
	100	12	114.7 ± 5.6	132.9 ± 6.8	145.7 ± 8.2	160.0 ± 10.6	184.5 ± 13.5	201.3 ± 14.5
	300	12	114.5 ± 4.9	131.7 ± 5.3	140.6 ± 5.3	156.2 ± 7.4	175.4 ± 9.5	189.7 ± 11.0
	Vehicle control	12	102.7 ± 3.7	116.1 ± 4.0	125.8 ± 5.5	135.5 ± 5.9	145.6 ± 7.6	153.2 ± 9.4
Female	10	6	103.2 ± 3.4	116.9 ± 4.9	128.5 ± 7.0	139.7 ± 7.3	150.1 ± 9.7	160.7 ± 8.6
	30	6	102.7 ± 4.9	115.6 ± 7.6	126.7 ± 7.7	135.0 ± 10.4	146.8 ± 12.3	153.2 ± 13.1
	100	12	102.7 ± 4.3	115.6 ± 3.8	124.6 ± 4.7	134.8 ± 5.8	145.7 ± 7.5	153.4 ± 6.7
	300	12	102.9 ± 4.4	115.9 ± 6.1	122.0 ± 7.6	133.4 ± 8.9	146.2 ± 11.2	154.7 ± 12.1
	Vehicle control	12	102.7 ± 3.7	116.1 ± 4.0	125.8 ± 5.5	135.5 ± 5.9	145.6 ± 7.6	153.2 ± 9.4
	Vehicle control	12	102.7 ± 3.7	116.1 ± 4.0	125.8 ± 5.5	135.5 ± 5.9	145.6 ± 7.6	153.2 ± 9.4

Mean ± S.D.

* : Significantly different from Vehicle control at P<0.05.
** : Significantly different from Vehicle control at P<0.01.

B11-0394
Table 2 -Continued
Mean body weights(g)

Sex	Exp.-group (ug/kg/day)	Number of animals	Administration					
			15	17	19	22	24	28 (days)
Male	Vehicle control	12	230.2 ± 18.4	243.7 ± 21.2	256.4 ± 21.3	275.5 ± 23.3	288.3 ± 23.8	298.5 ± 26.4
	10	6	241.5 ± 19.2	255.3 ± 19.3	269.3 ± 20.7	291.3 ± 21.3	304.9 ± 23.1	314.7 ± 23.0
	30	6	233.0 ± 14.4	248.6 ± 15.7	260.2 ± 15.7	281.0 ± 21.5	284.0 ± 21.8	303.9 ± 24.2
	100	12	240.3 ± 17.6	256.4 ± 18.3	270.8 ± 19.7	291.0 ± 22.0	304.7 ± 22.9	313.8 ± 24.2
	300	12	222.6 ± 13.1	236.3 ± 14.8	247.1 ± 16.7	263.8 ± 17.6	274.6 ± 18.0	281.4 ± 20.2
	Vehicle control	12	167.9 ± 11.5	175.9 ± 10.6	182.5 ± 14.1	190.3 ± 14.6	196.8 ± 14.4	201.0 ± 15.0
Female	10	6	180.7 ± 9.2	189.8 ± 6.8	195.7 ± 13.1	205.9 ± 10.9	214.8 ± 13.1	224.6 ± 11.3
	30	6	171.3 ± 14.5	179.4 ± 16.9	185.6 ± 15.3	191.7 ± 14.9	200.3 ± 16.3	209.2 ± 15.0
	100	12	171.1 ± 8.6	177.6 ± 9.5	185.4 ± 11.2	193.3 ± 11.8	199.6 ± 13.4	208.4 ± 10.5
	300	12	172.3 ± 15.3	180.4 ± 16.2	185.9 ± 16.6	193.8 ± 18.8	199.8 ± 19.0	208.5 ± 19.2
	Vehicle control	12	167.9 ± 11.5	175.9 ± 10.6	182.5 ± 14.1	190.3 ± 14.6	196.8 ± 14.4	201.0 ± 15.0
	Vehicle control	12	167.9 ± 11.5	175.9 ± 10.6	182.5 ± 14.1	190.3 ± 14.6	196.8 ± 14.4	201.0 ± 15.0

Mean ± S.D.

* : Significantly different from Vehicle control at P<0.05.

** : Significantly different from Vehicle control at P<0.01.

Table 2 -Continued
Mean body weights(g)

Sex	Exp. group (mg/kg/day)	Number of animals	Recovery						
			1	3	5	8	10	12	14 (days)
Male	Vehicle control	6	320.0 ± 33.2	332.3 ± 35.4	341.9 ± 34.5	357.1 ± 36.2	368.4 ± 37.4	377.2 ± 37.0	389.0 ± 37.9
	100	6	336.9 ± 32.7	345.6 ± 33.2	353.2 ± 34.9	371.0 ± 37.4	382.0 ± 37.8	388.8 ± 37.4	402.3 ± 39.4
	300	6	299.5 ± 14.4	306.8 ± 16.0	308.7 ± 19.6	323.7 ± 16.6	333.5 ± 18.9	341.7 ± 22.2	355.9 ± 19.8
	Vehicle control	6	212.6 ± 18.7	217.9 ± 17.1	220.5 ± 13.5	226.2 ± 18.8	228.0 ± 18.6	233.3 ± 22.6	235.5 ± 20.1
Female	100	6	212.0 ± 12.3	216.0 ± 12.1	219.9 ± 15.1	221.0 ± 15.6	228.4 ± 8.9	230.2 ± 18.1	236.1 ± 12.0
	300	6	205.4 ± 19.2	210.5 ± 18.6	212.2 ± 19.7	217.7 ± 22.6	219.1 ± 22.4	225.2 ± 21.8	228.4 ± 23.0

Mean ± S.D.

* : Significantly different from Vehicle control at P<0.05.

** : Significantly different from Vehicle control at P<0.01.

Table 3 28-day repeated-dose oral toxicity study in rats
Mean food consumption(g/rat/day)

Sex	Exp. group (mg/kg/day)	Number of animals	Administration							
			1	4	8	11	15	18	22	25
Male	Vehicle control	12	17.0 ± 1.7	17.2 ± 1.7	18.0 ± 1.6	17.8 ± 2.1	18.0 ± 2.3	16.9 ± 2.3	18.9 ± 2.0	17.6 ± 1.9
	10	6	17.2 ± 1.2	16.6 ± 1.9	18.7 ± 1.8	19.0 ± 2.5	19.6 ± 2.2	18.0 ± 1.9	19.1 ± 2.3	18.2 ± 2.3
	30	6	17.0 ± 1.2	16.6 ± 0.9	18.7 ± 1.4	18.3 ± 1.8	18.6 ± 1.3	17.5 ± 1.3	18.2 ± 2.0	18.0 ± 1.8
	100	12	17.5 ± 1.8	16.5 ± 2.0	19.5 ± 2.3	19.3 ± 2.1	18.8 ± 2.1	18.6 ± 1.8	19.9 ± 1.8	19.3 ± 1.9
	300	12	17.0 ± 1.0	15.6 ± 1.4	18.2 ± 1.4	17.9 ± 1.4	18.1 ± 1.8	17.3 ± 1.7	18.2 ± 1.6	17.8 ± 1.6
	Vehicle control	12	14.5 ± 1.0	14.0 ± 1.1	13.0 ± 1.2	13.0 ± 1.4	12.8 ± 1.0	12.7 ± 1.3	14.2 ± 1.6	13.4 ± 1.5
Female	Vehicle control	10	15.2 ± 0.8	14.7 ± 1.6	14.9 ± 1.3	14.3 ± 1.7	14.7 ± 1.6	14.3 ± 1.4	14.4 ± 2.0	14.4 ± 2.1
	30	6	15.0 ± 2.0	14.6 ± 1.7	14.5 ± 1.5	14.0 ± 1.7	13.3 ± 1.5	13.6 ± 1.5	14.0 ± 1.4	13.9 ± 1.4
	100	12	15.0 ± 0.8	13.6 ± 1.7	14.6 ± 1.8	13.6 ± 1.1	13.5 ± 1.4	12.7 ± 1.5	13.7 ± 1.5	13.6 ± 1.8
	300	12	14.3 ± 1.2	13.3 ± 1.8	14.6 ± 1.7	14.3 ± 1.9	13.5 ± 1.9	13.2 ± 1.8	13.8 ± 1.8	13.6 ± 2.1
	Vehicle control	10	15.0 ± 0.8	14.7 ± 1.7	14.5 ± 1.8	14.3 ± 1.7	14.0 ± 1.7	13.7 ± 1.5	13.7 ± 1.5	13.5 ± 1.1
	300	12	14.3 ± 1.2	13.3 ± 1.8	14.6 ± 1.7	14.3 ± 1.9	13.5 ± 1.9	13.2 ± 1.8	13.8 ± 2.0	13.7 ± 2.0

Mean ± S.D.

* : Significantly different from Vehicle control at P<0.05.

** : Significantly different from Vehicle control at P<0.01.

Table 3 -Continued
Mean food consumption(g/rat/day)

Sex	Exp. group (mg/kg/day)	Number of animals	Recovery			14 (days)
			4	8	11	
Male	Vehicle control	6	21.8	23.7	25.0	26.3
	100	6	21.4	3.4	3.2	3.2
	100	6	21.3	25.1	26.1	27.6
	300	6	18.4	3.6	3.1	3.9
Female						
	300	6	1.6	21.6	22.8	24.8
	Vehicle control	6	17.1	18.4	18.1	19.3
	100	6	1.1	1.3	1.8	2.4
	100	6	16.4	18.2	19.6	19.1
	300	6	1.0	1.4	1.7	1.5
	300	6	16.4	17.8	18.2	18.7
	300	6	2.0	2.6	3.3	2.8

Mean \pm S.D.

Figures in parentheses indicate number of animals used for mean calculation.

* : Significantly different from Vehicle control at P<0.05.

** : Significantly different from Vehicle control at P<0.01.

Table 4 28-day repeated-dose oral toxicity study in rats
Hematology

Sex	Exp.-group (mg/kg/day)	Number of animals	RBC (x10 ⁶ /mm ³)	WBC (x10 ⁴ /mm ³)	Hb (g/dl)	Ht (%)	MCV (μ m ³)	MCH (pg)	MCHC (%)	Platelet (x10 ⁴ /mm ³)	Reticulo (%)	P.T. (sec)	APTT (sec)
	Vehicle control	6	739	115	14.9	43.3	58.6	20.1	34.4	118.1	31	12.3	24.6
	10	6	712	118	14.2*	41.3*	58.0	20.0	34.4	116.3	30	12.5	23.7
		10	726	132	10.4	11.0	11.7	10.7	10.6	111.5	14	10.3	11.1
	30	6	734	103	14.4	42.3	57.7	19.7	34.1	107.2	29	12.3	24.7
		30	731	132	10.4	11.4	11.8	10.6	10.5	111.7	17	10.4	14.9
	100	6	757	103	15.2	44.5	58.8	20.1	34.2	114.6	33	12.7	24.8
		100	737	24	10.4	11.4	12.4	10.9	10.8	112.6	14	10.9	11.9
	300	6	756	75	15.4	45.1	59.6	20.3	34.1	96.7**	32	13.6	27.7
		300	717	18	10.4	11.3	11.5	10.5	10.3	119.2	19	11.6	12.2
	Recovery												
	Vehicle control	6	770	114	15.0	42.5	55.2	19.6	35.4	118.9	22	12.2	26.7
	10	6	738	113	10.3	11.4	11.4	10.8	10.6	118.5	110	10.7	15.2
		10	752	117	14.9	42.3	56.3	19.8	35.3	111.3	28	12.7	24.7
	100	6	734	30	10.5	11.3	12.6	10.8	10.5	110.8	111	10.4	12.0
		100	783	109	15.1	42.6	54.5	19.3	35.4	112.0	20	12.5	25.5
	300	6	737	27	10.4	10.7	12.4	10.9	10.6	117.4	19	10.8	12.3
		300	724	6	10.4	11.2	11.4	10.5	10.3	113.4	18	10.3	13.8
	Recovery												
	Vehicle control	6	733	66	14.9	42.1	57.4	20.3	35.4	125.1	22	11.7	21.7
	10	6	731	77	14.9	42.6	58.3	20.4	35.0	118.7	17	11.8	23.1
		10	732	25	10.5	11.8	10.9	10.3	10.4	114.7	16	10.5	11.7
	30	6	738	63	15.2	43.4	58.8	20.6	35.0	113.8	24	11.9	21.5
		30	717	22	10.4	11.7	12.4	10.6	10.9	13.9	13	10.5	12.4
	100	6	735	70	14.8	42.5	57.9	20.2	34.9	110.1	27	12.3	23.0
		100	725	7	10.2	11.5	11.4	10.5	10.8	112.5	14	10.3	12.0
	300	6	724	89	15.2	43.2	59.8	21.0*	35.2	107.6	28	12.1	21.6
		300	725	6	10.4	11.2	11.4	10.5	10.3	113.4	18	10.3	13.8
	Recovery												
	Vehicle control	6	777	84	15.6	43.1	55.4	20.1	36.2	118.5	17	12.0	19.8
	100	6	768	75	15.0	42.1	54.8	19.6	35.8	121.0	13	12.4	18.0
		100	734	21	10.3	11.5	11.9	10.8	10.9	117.1	13	10.3	12.2
	300	6	772	65	15.4	42.5	55.1	20.0	36.2	117.4	12	12.4	18.5
		300	726	11	10.4	10.7	11.4	10.4	10.5	115.9	14	10.2	12.2

Mean ± S.D.

* : Significantly different from Vehicle control at P<0.05.
** : Significantly different from Vehicle control at P<0.01.

Table 4 -Continued
Hematology

Sex	Rep. Group (mg/kg/day)	Number of animals	Differentiation of Leukocyte (%)					
			N-Band	N-Seg	Rosino	Beso	Lymph	
Male	Vehicle control	6	0.1	16.4	0.8	0.0	82.8	0.0
	10	6	0.3	4.2	11.0	10.0	4.5	10.0
	30	6	0.3	23.8	0.8	0.0	75.2	0.0
	100	6	0.4	20.4	0.7	0.0	78.6	0.0
	300	6	0.2	13.7	0.3	0.0	85.5	0.1
	Recovery	6	0.0	12.4	0.3	0.0	87.1	0.0
	Vehicle control	6	0.0	13.7	0.3	0.0	85.8	0.3
	100	6	0.0	12.5	10.3	10.0	4.2	10.4
	300	6	0.0	14.9	0.8	0.0	84.3	0.1
	Recovery	6	0.0	15.8	10.3	10.0	5.8	10.2
Female	Vehicle control	6	0.4	16.4	0.6	0.0	82.4	0.2
	10	6	0.3	16.6	0.7	0.0	82.3	0.1
	30	6	0.1	15.5	0.3	0.0	84.0	0.2
	100	6	0.2	12.9	0.4	10.0	3.1	10.4
	300	6	0.2	16.4	0.5	0.0	82.9	0.0
	Recovery	6	0.2	12.6	0.6	0.0	86.5	0.2
	Vehicle control	6	0.1	11.2	0.7	0.0	87.6	0.5
	100	6	0.1	11.5	1.6	10.0	4.6	10.8
	300	6	0.0	13.6	1.0	10.0	4.0	10.4
	Recovery	6	0.0	10.1	0.9	10.0	88.5	0.5

Mean ± S.D.

* : Significantly different from Vehicle control at P<0.05.

** : Significantly different from Vehicle control at P<0.01.

Table 5 28-day repeated-dose oral toxicity study in rats
Blood chemistry

Sex	Exp.-group (mg/kg/day)	Number of animals	GOT (IU/l)	GPT (IU/l)	ALP (IU/l)	ChE (IU/l)	γ -GTP (IU/l)	T-Chol (mg/dl)	TG (mg/dl)	Glucose (mg/dl)	T-protein (g/dl)	Albumin (g/dl)	A/G ratio	
	Vehicle control	6	60	16	452	48	0.6	74	116	130.8	5.9	2.9	0.98	
	10	6	64	14	512	10	10.3	7	23	8.6	10.1	10.1	10.06	
	30	6	57	15	452	57	0.6	74	144	136.7	5.8	2.8	0.96	
	100	6	57	17	611	12	10.2	12	80	156	135.4	5.9	2.8	0.93
	300	6	67	25*	716*	48	0.5	88	196	151.2	6.0	3.0	0.96	
	Recovery													
	Vehicle control	6	61	20	328	67	0.2	78	109	124.8	6.1	2.8	0.88	
	10	6	68	21	273	58	0.3	80	118	136.2	6.0	2.8	0.87	
	30	6	70	24*	293	72	0.3	79	103	124.0	6.0	2.8	0.94	
	100	6	77	2	164	33	0.1	13	49	127.1	5.9	2.8	0.92	
	300	6	90**	14	395	125**	0.9	112**	111*	103.7	6.2	3.2	1.03	
	Recovery													
	Vehicle control	6	59	13	263	266	0.7	66	36	120.6	6.1	3.0	1.00	
	10	6	58	14	272	100	0.2	12	6	16.0	10.2	10.1	10.04	
	30	6	61	14	292	262	0.7	66	37	130.3	6.3	3.1	0.99	
	100	6	73	15	304	191	0.7	80	52	123.5	6.2	3.2	1.02	
	300	6	77	16	215	61	0.2	4	18	14.0	10.2	10.2	10.05	
	Recovery													
	Vehicle control	6	76	18	164	342	0.6	76	62	124.6	6.4	3.2	0.98	
	10	6	76	19	166	313	0.5	82	49	125.1	6.2	3.0*	0.93	
	30	6	77	16	215	336	0.5	87	48	127.1	6.5	3.1	0.92	
	100	6	112	3	71	81	0.2	12	13	10.6	10.2	10.1	10.05	

Mean \pm S.D.

* : Significantly different from Vehicle control at P<0.05.
** : Significantly different from Vehicle control at P<0.01.

Table 5 -Continued
Blood chemistry

Sex	Exp.-group (mg/kg/day)	Number of animals	BUN (mg/dl)	Creatinine (mg/dl)	T-Bil (mg/dl)	Ca (mg/dl)	IP (mEq/dl)	Na (mEq/l)	K (mEq/l)	C1 (mEq/l)
	Vehicle control	6	9.2 ± 1.2	0.45 ± 0.04	0.25 ± 0.02	9.8 ± 0.2	7.4 ± 0.4	143 ± 1	4.2 ± 0.3	105.7 ± 1.7
Male	10	6	8.9 ± 0.9	0.45 ± 0.05	0.24 ± 0.04	9.9 ± 0.2	8.0 ± 0.2	144 ± 1	4.3 ± 0.3	106.8 ± 1.2
	30	6	8.5 ± 1.4	0.45 ± 0.05	0.26 ± 0.06	9.9 ± 0.3	8.2* ± 0.5	143 ± 1	4.3 ± 0.3	105.8 ± 1.2
	100	6	8.7 ± 1.6	0.51 ± 0.06	0.25 ± 0.03	10.2 ± 0.4	8.5** ± 0.5	144 ± 1	3.9 ± 0.3	104.7 ± 2.5
	300	6	8.5 ± 0.8	0.44 ± 0.16	0.33 ± 0.08	10.0 ± 0.4	8.5** ± 0.7	144 ± 1	4.0 ± 0.2	106.8 ± 0.7
	Recovery									
Vehicle control	6	14.0 ± 1.4	0.42 ± 0.05	0.25 ± 0.05	10.1 ± 0.3	7.1 ± 0.4	144 ± 1	4.2 ± 0.3	105.0 ± 1.2	
	100	6	15.3 ± 1.3	0.47 ± 0.07	0.21 ± 0.02	10.1 ± 0.2	7.4 ± 0.6	144 ± 0	4.0 ± 0.3	104.6 ± 0.8
	300	6	14.3 ± 1.3	0.44 ± 0.10	0.24 ± 0.03	10.2 ± 0.4	7.5 ± 0.7	145 ± 1	4.1 ± 0.3	104.8 ± 1.0
Vehicle control	6	11.5 ± 2.4	0.50 ± 0.02	0.21 ± 0.01	10.0 ± 0.2	7.0 ± 0.6	142 ± 1	4.2 ± 0.2	107.5 ± 1.8	
	10	6	10.7 ± 1.3	0.48 ± 0.10	0.24 ± 0.05	10.1 ± 0.2	7.6 ± 0.7	142 ± 1	4.1 ± 0.6	106.9 ± 1.0
	30	6	10.1 ± 1.2	0.48 ± 0.03	0.24 ± 0.02	10.1 ± 0.3	7.5 ± 0.8	143 ± 1	3.9 ± 0.1	108.4 ± 2.1
	100	6	11.7 ± 1.1	0.48 ± 0.03	0.25* ± 0.02	10.1 ± 0.2	7.5 ± 0.2	142 ± 1	4.3 ± 0.2	107.5 ± 1.7
Female	300	6	10.8 ± 1.4	0.44 ± 0.03	0.25** ± 0.02	10.0 ± 0.1	8.2** ± 0.4	143 ± 1	4.2 ± 0.3	107.3 ± 0.8
	Recovery									
Vehicle control	6	15.9 ± 1.5	0.55 ± 0.06	0.19 ± 0.06	9.9 ± 0.3	6.0 ± 0.5	144 ± 1	4.3 ± 0.6	108.6 ± 1.6	
	100	6	17.5 ± 2.4	0.52 ± 0.07	0.21 ± 0.04	10.0 ± 0.2	6.4 ± 0.4	144 ± 1	4.3 ± 0.3	107.4 ± 1.0
	300	6	16.5 ± 1.4	0.55 ± 0.04	0.19 ± 0.03	10.0 ± 0.3	6.5 ± 0.6	144 ± 1	4.2 ± 0.2	107.4 ± 1.0

Mean ± S.D.

* : Significantly different from Vehicle control at P<0.05.

** : Significantly different from Vehicle control at P<0.01.

Table 6 28-day repeated-dose oral toxicity study in rats
Urinalysis

B11-0394

Sex	Exp. group (mg/kg/day)	Number of animals	Volume ^{a)} (ml)	Color			pH	Protein	ketones	Bilirubin	Occult Blood	Glucose	Urobilinogen (EU/dl)
				SY	Y	SB							
	Vehicle control	6	9	2	3	1	1	4	0	0	1	3	2
	10	6	9	2	4	0	0	1	5	0	0	2	3
	30	6	10	2	3	1	2	2	0	0	2	2	1
Male	100	6	12	3	3	0	0	2	4	0	0	3	2
	300	6	19*	5	1	0	1	1	4	0	0	5	0
			± 8										
	Recovery												
	Vehicle control	6	12	3	3	0	0	0	3	3	0	3	1
	100	6	12	3	3	0	0	1	4	1	0	3	1
	300	6	15	4	2	0	0	0	4	2	0	5	0
			± 4										
	Vehicle control	6	9	2	4	0	0	1	5	0	0	4	1
	10	6	10	4	2	0	0	0	6	0	0	5	1
	30	6	10	2	4	0	0	2	4	0	0	4	2
Female	100	6	12	4	2	0	0	1	5	0	0	5	0
	300	6	17**	6	0	0	0	2	4	0	1	5	0
			± 5										
	Recovery												
	Vehicle control	6	16	5	1	0	0	0	3	3	2	3	1
	100	6	12	2	4	0	0	0	6	0	0	2	2
	300	6	14	5	1	0	0	0	3	3	1	5	0
			± 5										

a) Mean ± S.D.

* : Significantly different from Vehicle control at P<0.05.

** : Significantly different from Vehicle control at P<0.01.

SY : Slightly yellow

Y : Yellow

Table 7 28-day repeated-dose oral toxicity study in rats
Absolute organ weights

BII-0394

Sex	Rin group (mg/kg/day)	Number of animals	Spleen (g)	Liver (g)	Kidney (g)	Brain (g)	Testis (g)	Adrenal gland (mg)	Ovary (mg)	Body weight (g)
Male	Vehicle control	6	0.52	8.75	2.03	1.87	2.51	38.8	-	287.1
	10	6	0.04	0.72	0.20	0.13	0.14	5.4	-	19.0
	30	6	0.55	10.12	2.25	0.03**	2.91**	40.6	-	310.8
	100	6	0.05	11.35	0.31	0.08	0.21	3.7	-	23.1
	300	6	0.55	10.10	2.25	1.92	8.3*	44.8	-	298.1
	Vehicle control	6	0.10	11.38	0.27	0.08	0.09	7.4	-	26.6
	100	6	0.51	11.30**	2.20	1.88	2.75	46.0	-	305.8
	300	6	0.09	11.25	0.18	0.04	0.24	8.1	-	18.1
	Recovery	6	0.39*	11.38**	2.08	1.82	2.69	42.2	-	266.0
	Vehicle control	6	0.05	11.50	0.22	0.07	0.21	5.0	-	24.3
Female	Vehicle control	6	0.68	10.65	2.50	1.98	3.10	50.0	-	364.0
	10	6	0.11	1.50	0.33	0.05	0.26	7.7	-	35.9
	30	6	0.69	11.03	2.58	1.93	2.86	44.7	-	377.7
	100	6	0.05	11.30	0.18	0.03	0.38	17.6	-	37.2
	300	6	0.58	9.84	2.36	1.94	2.94	45.8	-	330.7
	Vehicle control	6	0.35	5.79	1.33	1.81	-	47.7	74.3	192.6
	10	6	0.46*	6.85*	1.50	0.14	0.08	5.4	9.5	9.1
	30	6	0.34	6.62	1.53	1.79	-	53.9	75.6	212.2
	100	6	0.35	6.43	0.12	0.08	-	5.1	6.9	14.3
	300	6	0.07	6.66	1.47	1.75	-	13.0	15.5	14.3
	Recovery	6	0.39	8.15**	0.21	0.06	-	47.5	82.6	198.5
	Vehicle control	6	0.05	0.59	0.13	0.09	-	6.3	8.8	16.8

Mean \pm S.D.

* : Significantly different from Vehicle control at P<0.05.

** : Significantly different from Vehicle control at P<0.01.

Table 8 28-day repeated-dose oral toxicity study in rats
Relative organ weights

Sex	Bip. group (mg/kg/day)	Number of animals	Spleen (g/100g)	Liver (g/100g)	Kidney (g/100g)	Brain (g/100g)	Testis (g/100g)	Adrenal gland (mg/100g)	Ovary (mg/100g)	Body weight (g)
Male	Vehicle control	6	0.18 ±0.02	3.05 ±0.11	0.71 ±0.05	0.65 ±0.06	0.88 ±0.08	13.5 ±1.4	-	287.1 ±19.0
	10	6	0.18 ±0.01	3.25 ±0.28	0.72 ±0.07	0.66 ±0.06	0.94 ±0.04	13.1 ±0.6	-	310.8 ±23.1
	30	6	0.19 ±0.03	3.38* ±0.19	0.76 ±0.05	0.65 ±0.05	0.96 ±0.11	15.1 ±2.7	-	298.1 ±26.6
	100	6	0.17 ±0.03	3.69** ±0.22	0.72 ±0.04	0.62 ±0.04	0.90 ±0.06	15.0 ±2.2	-	305.8 ±18.1
	300	6	0.15* ±0.02	4.27** ±0.20	0.78 ±0.03	0.69 ±0.04	1.02 ±0.11	15.8 ±1.0	-	266.0 ±24.3
	Recovery	6	0.19 ±0.03	2.92 ±0.23	0.89 ±0.04	0.55 ±0.05	0.85 ±0.06	13.7 ±1.0	-	364.0 ±35.9
Female	Vehicle control	6	0.19 ±0.02	2.92 ±0.17	0.69 ±0.05	0.52 ±0.06	0.77 ±0.14	11.8 ±4.4	-	377.7 ±37.2
	100	6	0.19 ±0.02	2.92 ±0.17	0.69 ±0.05	0.52 ±0.06	0.77 ±0.14	11.8 ±4.4	-	330.7 ±20.4
	300	6	0.17 ±0.03	2.96 ±0.21	0.72 ±0.04	0.59 ±0.05	0.89 ±0.09	14.0 ±3.9	-	330.7 ±20.4
	Vehicle control	6	0.18 ±0.02	3.00 ±0.16	0.69 ±0.03	0.94 ±0.04	-	24.7 ±2.2	38.8 ±6.0	192.6 ±9.1
	10	6	0.21 ±0.03	3.22 ±0.17	0.71 ±0.02	0.85 ±0.05	-	25.6 ±3.9	35.9 ±5.3	212.2 ±14.3
	30	6	0.17 ±0.03	3.32** ±0.06	0.77** ±0.04	0.90 ±0.04	-	24.7 ±5.5	37.9 ±5.1	199.3 ±14.3
Female	100	6	0.18 ±0.03	3.35** ±0.19	0.74 ±0.06	0.88 ±0.08	-	23.8 ±1.4	41.9 ±6.8	198.5 ±16.8
	300	6	0.19 ±0.01	4.02** ±0.15	0.79** ±0.03	0.89 ±0.05	-	24.8 ±1.1	38.2 ±3.3	202.9 ±17.6
	Recovery	6	0.22 ±0.02	2.81 ±0.15	0.68 ±0.08	0.83 ±0.05	-	26.6 ±5.4	42.7 ±10.5	219.9 ±19.7

Mean ±S.D.

* : Significantly different from Vehicle control at P<0.05.

** : Significantly different from Vehicle control at P<0.01.

Table 9 28-day repeated-dose oral toxicity study in rats
Gross pathological findings

B11-0394

Findings	Male						Female					
	Vehicle	Vehicle		Vehicle		(Recovery)	(Recovery)	Vehicle		Vehicle		(Recovery) (mg/kg/day)
		control	10	30	100			control	10	30	100	
	ta*	ta	ta	ta	ta	ta	ta	ta	ta	ta	ta	ta
	6**	6	6	6	6	6	6	6	6	6	6	6
Liver												
Enlargement		0	0	0	0	0	5	0	0	0	0	0
Glandular stomach												
Blackish region of mucosa		0	0	0	0	0	1	1	0	0	1	0
Testis												
Scallop		0	0	0	0	0	1	0	0	0	1	0
Oral cavity												
Swelling of gingiva		0	0	0	0	0	0	1	0	0	0	0
Blackish region of incisor		0	0	0	0	0	0	6	0	0	0	6

* ta, terminal autopsy.

** Number of animals examined.

Table 10 28-day repeated-dose oral toxicity study in rats
Histopathological findings

Findings	Male						Female					
	Vehicle		Vehicle		Vehicle		Vehicle		Vehicle		Vehicle	
	control	control	10	30	100	300	control	control	10	30	100	300
ta*	ta	ta	ta	ta	ta	ta	ta	ta	ta	ta	ta	(Recovery) (mg/kg/day)
Grade	6**	6	6	6	6	6	6	6	6	6	6	6
Liver												
No abnormalities detected	6/6***	6/6	-	6/6	4/6	6/6	0/6	5/6	6/6	6/6	-	0/6
Ground glass appearance of hepatocytes	+ ++	0/6 0/6	0/6 0/6	-	0/6 0/6	0/6 0/6	4/6 2/6	0/6	0/6 0/6	0/6 0/6	-	0/6 0/6
Microvesicular steatosis of hepatocytes	+	0/6	0/6	-	0/6 0/6	0/6 0/6	0/6 0/6	1/6	0/6	0/6	-	0/6
Prominent nucleoli of hepatocytes	+	0/6	0/6	-	0/6 0/6	0/6 0/6	6/6 6/6	0/6	0/6	0/6	-	0/6
Swelling of hepatocytes	+	0/6	0/6	-	0/6 0/6	2/6 0/6	0/6 0/6	0/6	0/6	0/6	-	0/6
Spleen												
No abnormalities detected	6/6	-	-	-	-	6/6	-	6/6	-	-	-	6/6
Kidney												
No abnormalities detected	5/6	-	-	-	-	-	3/6	-	5/6	-	-	6/6
Increased eosinophilic bodies	± + ++	0/6 0/6 1/6	-	-	-	-	2/6 1/6 0/6	-	0/6	-	-	0/6
Mineralization in corticomedullary junction	+	0/6	-	-	-	-	0/6	-	0/6	-	-	0/6
Heart												
No abnormalities detected	6/6	-	-	-	-	6/6	-	6/6	-	-	-	6/6
Forestomach												
No abnormalities detected	6/6	-	-	-	-	6/6	-	6/6	-	-	-	6/6

* ta, terminal autopsy.

** Number of animals autopsied.

*** Number of animals affected / Number of animals examined.

- Not examined.

t, very slight; +, slight; ++, moderate; +++, severe.

Table 10 — Continued
Histopathological findings

B11-0394

Findings	Vehicle	Male						Female					
		control			Vehicle control			control			Vehicle control		
		10	30	100	300	300	(Recovery)	10	30	100	300	300	(Recovery)
	ta	ta	ta	ta	ta	ta	(Recovery)	ta	ta	ta	ta	ta	(Recovery)
Grade	6	6	6	6	6	6		6	6	6	6	6	
Glandular stomach													
No abnormalities detected	6/6	—	—	—	—	—	5/6	0/1	6/6	—	0/1	—	5/6
Necrosis of mucosa	+	0/6	—	—	—	—	1/6	1/1	0/6	—	1/1	—	1/6
Duodenum													
No abnormalities detected	6/6	—	—	—	—	—	6/6	—	6/6	—	—	—	6/6
Jejunum													
No abnormalities detected	6/6	—	—	—	—	—	6/6	—	6/6	—	—	—	6/6
Ileum													
No abnormalities detected	6/6	—	—	—	—	—	6/6	—	6/6	—	—	—	6/6
Cecum													
No abnormalities detected	6/6	—	—	—	—	—	6/6	—	6/6	—	—	—	6/6
Colon													
No abnormalities detected	6/6	—	—	—	—	—	6/6	—	6/6	—	—	—	6/6
Rectum													
No abnormalities detected	6/6	—	—	—	—	—	6/6	—	6/6	—	—	—	6/6
Testis													
Atrophy of seminiferous tubules	++	—	—	—	—	—	1/1	—	—	—	—	—	—
Interstitial cell hyperplasia	+	—	—	—	—	—	—	—	—	—	—	—	—
Adrenal													
No abnormalities detected	6/6	—	—	—	—	—	6/6	—	6/6	—	—	—	6/6
Incisor													
No abnormalities detected	—	6/6	—	—	—	—	6/6	—	6/6	—	—	—	6/6
Degeneration and irregular alignment of ameloblasts at stage of maturation	+	—	0/6	—	—	0/6	—	0/6	—	0/6	—	—	0/6
	+	—	0/6	—	—	0/6	—	6/6	—	0/6	—	—	0/6

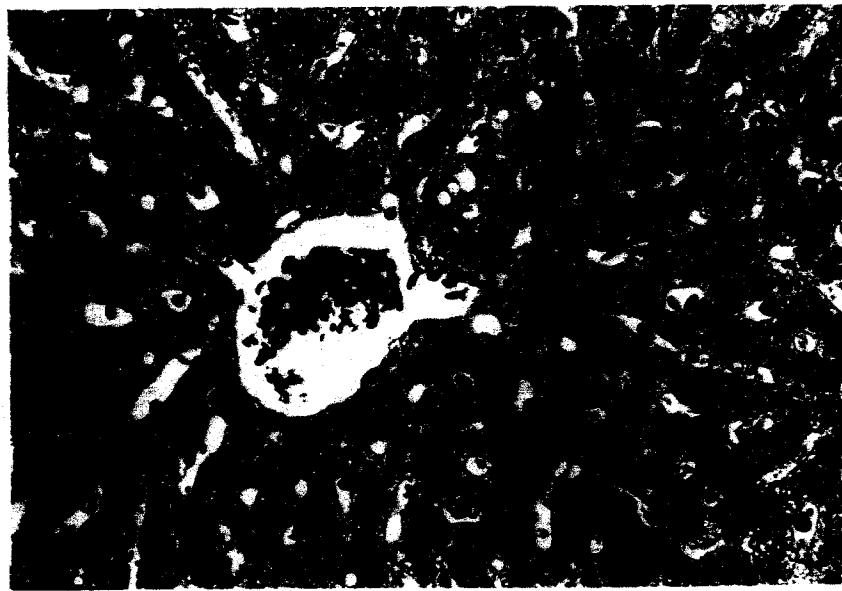


Photo. 1 Liver of a male rat given vehicle orally for 28 days.
Normal.
No. 1 animal. HE. $\times 360$.

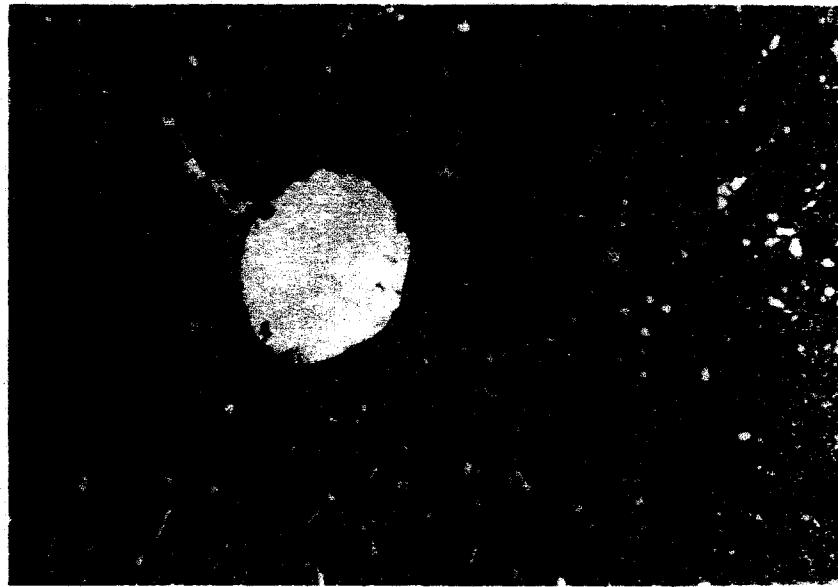


Photo. 2 Liver of a male rat given test substance at dose level of 300 mg/kg/day orally
for 28 days.
Swelling, ground glass appearance and prominent nucleoli of hepatocytes.
No. 39 animal. HE. $\times 360$.



Photo. 3 Incisor of a male rat given vehicle orally for 28 days (recovery group).

Normal.

No. 7 animal. HE. $\times 180$



Photo. 2 Incisor of a male rat given test substance at dose level of 300 mg/kg/day orally for 28 days (recovery group).

Degeneration and irregular alignment of ameloblasts at stage of maturation.

No. 47 animal. HE. $\times 180$

Addendum I 28-day repeated-dose oral toxicity study in rats
 Clinical signs
 Vehicle control

B11-0394

Signs	Sex	Administration				Recovery	
		1	2	3	4 (weeks)	1	2 (weeks)
No abnormalities detected	Male	1, 4, 6-10, 12*	4-7, 10	1, 2, 4-7, 11	1, 2, 4, 6, 7	7-12	7-12
	Female	49, 51-54, 56-59	49, 51, 53, 54, 56-60	49, 51, 53, 54, 56-60	49, 51, 53, 54, 56, 57, 58, 60	55-60	55-60
Salivation	Male	2, 3, 5, 11	1-2, 8, 9, 11, 12	3, 8-10, 12	3, 5, 8-12		
	Female	50, 55, 60	50, 52, 55	50, 52, 55	50, 52, 55, 58		

* Animal number.

+
Addendum 1 - Continued
Clinical signs
10 mg/kg/day

B11-0394

Signs	Sex	Administration				Recovery	
		1	2	3	4 (weeks)	1	2 (weeks)
No abnormalities detected	Male	14.16*	14.16	14.16	14.16		
	Female	61-66	62.65, 66	62.65, 66	62.65, 66	62.65, 66	62.65, 66
Salivation	Male	13.15, 17, 18	13.15, 17, 18	13.15, 17, 18	13.15, 17, 18	13.15, 17, 18	13.15, 17, 18
	Female		61.63, 64	61.63, 64	61.63, 64	61.63, 64	61.63, 64

* Animal number.

UT Addendum 1 - Continued
 Clinical signs
 30 mg/kg/day

B11-0394

Signs	Sex	Administration				Recovery	
		1	2	3	4 (weeks)	1	2 (weeks)
No abnormalities detected	Male Female	19-23* 67	67	67-72	67-69-72		
Salivation	Male Female	20-22, 24 68-72	68-72	19-24 67-68	19-24 68		

* Animal number.

57
Addendum 1 - Continued
Clinical signs
100 mg/kg/day

B11-0394

Signs	Sex	Administration				Recovery
		1	2	3	4 (weeks)	
No abnormalities detected	Male	25*	25	25	25, 28	31-34, 36
	Female	73, 77, 80	73, 80	77, 80	77, 80	79-84
Salivation	Male	26-36	26-36	26-36	26, 27, 29-36	31-34, 36
	Female	74-76, 78, 79, 81-84	74-79, 81-84	73-76, 78, 79, 81-84	73-76, 78, 79, 81-84	79-84
Exudate (neck)	Male					
	Female					
Loss of hair (neck)	Male				35	
	Female				35	
Scab formation (neck)	Male				35	
	Female				35	

* Animal number.

Addendum 1 - Continued
 Clinical signs
 300 mg/kg/day

B11-0394

Signs	Sex	Administration				Recovery	
		1	2	3	4 (weeks)	1	2 (weeks)
No abnormalities detected	Male						43, 44, 46-48*
	Female						91, 93-96
Salivation	Male	37-48		37-48		37-48	
	Female	85-96		85-96		85-96	
Decreased spontaneous locomotion	Male	37-46					
	Female	85, 86, 88, 93, 96					
Twisted region of incisor	Male						43-48
	Female						91-96
Loss of incisor (right upper)	Male					45	
	Female						
Swelling of gingiva	Male					45	
	Female						
Malocclusion	Male					45	
	Female						
Staining over abdomen	Male					89	
	Female						
Staining around anus	Male					92	
	Female						
Moist hair of lower abdomen	Male					92	
	Female						

* Animal number.

Addendum 2 28-day repeated-dose oral toxicity study in rats
Body weights(g)

B11-0394

Sex	Exp. group (mg/kg/day)	Animal No.	Administration					
			-2	1	3	5	8	10
♂		1	120.4	138.3	156.7	171.3	195.0	213.7
		2	116.8	136.2	147.7	163.7	179.9	190.7
		3	116.5	133.1	148.2	158.1	180.0	195.4
		4	110.1	130.3	140.9	152.7	174.7	185.0
		5	113.3	129.9	144.0	157.6	173.8	189.1
Vehicle control	6	108.0	122.2	137.2	150.8	169.2	183.1	193.7
	7	108.6	124.5	137.4	150.1	170.6	184.3	197.6
	8	114.3	132.0	143.6	154.5	173.5	186.3	197.9
	9	120.9	143.3	162.8	181.0	202.1	226.2	241.0
	10	120.3	145.1	158.2	177.8	201.9	219.7	236.4
	11	111.1	127.4	142.6	150.2	167.9	182.4	192.2
	12	112.6	128.9	144.1	160.4	180.5	195.9	210.9
	13	113.1	131.7	144.6	158.8	182.3	197.9	210.5
	14	109.3	124.4	133.8	145.7	161.8	175.3	184.6
♂	15	110.4	130.1	145.9	162.0	188.5	208.9	222.4
	16	115.1	132.2	152.1	163.0	183.4	201.9	216.9
	17	122.7	143.4	157.6	172.6	192.4	210.4	229.6
	18	117.4	133.5	153.7	170.6	193.7	212.5	227.1
	19	109.6	126.3	139.8	152.3	170.5	188.0	197.8
	20	108.9	126.8	141.5	154.8	177.6	194.4	209.8
	21	114.9	133.5	148.3	163.7	187.1	200.0	212.4
♂	22	124.1	142.1	158.1	172.7	197.5	217.1	229.5
	23	117.4	133.7	147.9	168.9	176.4	188.1	202.8
	24	112.1	129.2	143.7	160.1	184.3	200.9	213.4
	25	116.0	134.0	145.2	160.0	183.2	198.9	214.2
	26	117.3	134.0	148.8	163.7	190.0	208.2	223.2
	27	113.9	131.3	144.8	159.1	185.5	203.6	215.8
	28	124.7	147.6	159.6	178.5	204.2	220.5	233.5
	29	107.9	123.6	134.4	148.2	164.4	180.4	194.9
	30	111.6	127.5	140.8	160.3	174.1	188.3	203.5
♂	31	120.1	139.5	155.2	169.0	199.5	215.8	230.0
	32	115.0	131.1	145.9	158.8	181.2	198.5	211.3
	33	120.4	140.8	156.2	176.4	203.8	220.7	239.4
	34	114.2	129.4	136.5	148.9	166.3	179.8	195.6
	35	109.9	126.0	136.0	148.6	173.4	190.0	205.2
	36	104.9	128.4	144.4	159.3	188.7	210.4	225.7
	37	112.3	129.0	137.0	147.4	185.5	177.1	187.8
	38	106.3	121.6	130.5	139.8	155.9	166.0	177.7
	39	121.7	137.9	148.5	165.1	184.6	198.6	212.3
	40	118.8	134.8	145.2	162.5	186.0	200.2	216.5
	41	116.6	135.7	145.2	157.0	178.9	193.6	208.4
♂	42	111.3	130.0	137.0	151.8	168.7	183.1	196.4
	43	119.7	135.3	140.9	159.5	184.8	197.7	214.5
	44	109.1	126.0	135.6	149.9	166.8	179.4	195.2
	45	114.6	132.8	139.7	156.8	176.7	193.6	207.1
	46	120.3	139.0	145.9	164.2	184.0	201.4	213.8
	47	111.9	131.9	143.8	155.1	180.1	196.3	211.4
	48	111.6	127.2	138.0	153.8	173.0	189.5	201.6

Appendix 2 -Continued
Body weights(g)

B11-0394

Sex	Exp. group (mg/kg/day)	Animal No.	Administration						
			15	17	19	22	24	26	28 (days)
Vehicle control	1	248.6	264.5	274.3	300.2	313.5	326.2	335.9	
	2	224.5	230.3	239.3	259.6	269.3	274.4	285.2	
	3	234.0	249.6	264.6	287.1	299.2	305.3	321.0	
	4	215.1	227.0	240.9	254.7	264.8	276.0	282.6	
	5	223.1	232.9	247.5	263.1	278.9	286.6	293.6	
	6	218.3	229.8	243.6	261.8	273.4	283.2	289.5	
	7	218.0	228.3	244.3	259.6	277.0	284.1	295.0	
	8	217.1	226.5	235.1	253.1	263.9	273.0	280.8	
	9	286.3	282.5	295.1	313.5	326.1	342.1	354.9	
	10	258.9	279.0	292.6	313.4	328.8	345.6	355.7	
Male	11	210.0	224.0	236.8	254.3	269.7	280.1	289.9	
	12	230.5	250.2	262.8	285.3	296.7	305.8	316.7	
	13	236.0	252.4	263.6	287.5	299.2	305.6	316.2	
	14	205.7	218.7	230.8	250.0	260.5	271.5	281.9	
	15	249.7	262.4	275.0	298.3	315.1	323.9	338.6	
	16	244.7	258.2	275.2	301.3	315.8	329.5	341.8	
	17	258.1	273.1	289.2	306.9	322.0	330.8	342.4	
	18	254.5	266.8	281.8	303.5	316.9	326.7	339.7	
	19	218.9	230.2	238.4	252.9	265.6	272.7	280.2	
	20	232.6	249.4	265.3	292.7	304.8	315.2	330.1	
30	21	235.4	252.0	262.3	281.3	296.2	305.3	314.3	
	22	256.7	273.0	281.1	308.9	321.8	335.1	348.2	
	23	217.2	232.7	245.2	270.5	270.5	277.9	283.0	
	24	237.4	254.4	268.6	291.4	304.9	317.1	327.2	
	25	234.9	249.5	263.8	285.1	298.4	311.5	316.7	
	26	249.2	263.6	280.9	304.2	322.3	335.9	346.9	
	27	239.2	252.1	268.1	286.1	298.5	311.1	322.3	
	28	258.2	275.6	288.6	305.0	313.6	325.8	340.9	
	29	213.8	230.8	246.4	264.4	278.4	289.3	301.0	
	30	225.3	240.2	252.3	273.4	289.1	294.7	303.1	
100	31	259.0	275.8	290.8	312.7	327.8	334.3	345.1	
	32	232.6	251.0	265.8	282.2	294.8	307.1	317.6	
	33	268.2	286.8	301.2	327.0	339.2	354.5	364.5	
	34	216.0	229.6	236.6	252.7	261.3	269.5	277.2	
	35	232.1	251.1	266.6	285.4	302.5	314.7	328.6	
	36	265.2	271.2	288.5	314.1	329.9	337.2	353.1	
	37	206.2	214.9	224.2	238.1	247.2	251.8	261.9	
	38	195.5	208.4	213.4	228.7	237.6	243.7	249.8	
	39	224.9	238.4	249.7	262.9	272.3	278.3	282.0	
	40	240.0	255.8	266.4	284.5	298.9	308.4	320.9	
300	41	227.1	242.3	255.4	272.0	281.4	286.9	296.3	
	42	217.2	228.8	241.2	263.1	271.5	277.4	289.5	
	43	235.6	254.5	267.9	284.8	298.1	309.9	319.1	
	44	212.7	225.8	235.1	253.3	260.8	269.6	278.4	
	45	230.1	243.2	254.3	273.9	285.0	295.8	301.6	
	46	231.5	242.9	255.3	272.1	283.2	299.1	303.2	
	47	232.3	247.9	260.6	277.6	289.8	294.2	303.2	
	48	216.9	232.4	241.5	252.7	268.3	272.8	279.2	

Addendum 2 -Continued
Body weights(g)

B11-0394

60

Sex	Exp. group (mg/kg/day)	Animal No.	Administration				
			-2	1	3	5	8
Vehicle control	49	109.0	123.7	133.7	141.2	156.0	162.6
	50	101.2	114.1	121.1	130.2	139.5	147.7
	51	99.7	115.4	127.5	135.7	148.3	152.5
	52	97.6	112.1	117.1	126.7	136.6	144.8
	53	105.9	121.0	127.9	131.8	139.0	140.8
	54	103.7	112.9	127.1	136.6	147.1	150.5
	55	102.9	118.1	129.1	140.2	156.8	165.0
	56	98.1	112.0	121.8	132.8	137.9	144.2
	57	107.6	120.2	131.2	144.3	153.1	168.8
	58	105.0	118.3	132.8	144.3	152.8	164.1
Female	59	99.9	112.6	120.3	130.9	140.0	149.8
	60	101.4	113.2	120.3	130.8	140.1	147.9
	61	101.0	115.2	126.8	138.6	152.3	159.6
	62	106.2	117.2	129.4	140.9	153.3	162.2
	63	98.6	112.6	121.9	136.5	145.3	157.7
	64	107.9	126.4	141.8	153.3	167.0	176.7
	65	103.5	114.9	124.1	132.3	140.7	151.4
	66	101.9	115.1	127.0	136.4	142.2	156.5
	67	107.3	120.4	132.8	138.2	153.1	157.4
	68	103.0	115.3	127.1	136.9	148.1	150.7
Male	69	95.3	103.2	115.7	123.0	133.2	142.9
	70	99.4	111.9	121.1	132.5	132.8	134.9
	71	102.3	117.6	126.3	137.9	148.4	162.3
	72	108.6	124.9	137.2	150.5	165.1	170.9
	73	102.6	117.8	119.8	129.1	140.2	149.5
	74	104.7	118.5	129.8	141.5	156.6	166.0
	75	97.2	109.3	117.7	123.6	141.0	141.3
	76	100.4	115.0	120.3	130.0	138.6	146.2
	77	101.5	114.2	125.2	134.4	144.7	154.7
	78	110.5	121.7	128.1	139.6	146.3	155.1
300	79	95.8	110.9	124.1	141.5	162.2	162.5
	80	106.1	116.1	130.5	139.0	146.5	153.5
	81	108.4	120.5	131.5	140.2	149.4	156.0
	82	99.3	112.8	119.1	131.4	138.3	151.4
	83	103.9	117.3	126.3	137.0	145.9	155.2
	84	101.6	112.6	122.8	130.5	138.2	149.3
	85	100.0	112.6	120.2	134.2	148.6	154.5
	86	104.1	118.3	125.3	142.4	148.6	164.1
	87	104.4	120.8	128.1	138.8	158.5	164.4
	88	101.2	112.2	119.2	131.9	142.5	152.0
300	89	111.0	127.7	137.0	146.7	165.1	174.3
	90	96.5	107.4	112.1	120.9	131.9	140.3
	91	98.5	110.1	113.6	121.7	133.6	137.6
	92	101.8	113.9	116.5	126.7	138.8	149.0
	93	103.3	119.2	122.9	135.8	144.9	150.6
	94	109.6	122.3	131.0	144.4	161.2	170.8
	95	106.1	117.5	124.4	134.2	148.5	158.7
	96	98.8	108.7	113.8	122.5	131.8	140.0

Addendum 2 -Continued
Body weights(g)

B11-0394

Sex	Exp. group (mg/kg/day)	Animal No.	Administration					
			15	17	19	21	24	26
Vehicle control	49	179.1	177.9	191.4	197.0	205.1	205.4	212.5
	50	158.1	170.3	169.6	178.4	182.8	187.9	189.6
	51	172.2	177.8	183.4	193.7	200.7	205.0	212.6
	52	156.9	166.3	171.9	180.5	187.5	193.0	196.1
	53	155.1	165.4	167.3	170.9	183.5	181.2	192.1
	54	163.5	174.5	181.7	189.5	196.2	202.6	213.3
	55	182.9	194.2	201.1	202.7	215.7	221.1	223.5
	56	158.6	166.9	170.8	176.9	185.8	190.7	195.0
	57	182.5	196.1	209.6	225.7	224.0	231.2	226.6
	58	184.7	183.1	198.8	196.9	211.4	213.6	217.4
Female	59	161.1	171.0	175.7	182.7	182.7	189.7	190.3
	60	160.4	166.7	171.2	184.7	186.3	190.2	194.1
	61	181.5	191.5	198.9	207.8	224.5	220.9	236.3
	62	177.0	187.2	199.7	213.8	220.9	224.8	231.3
	63	175.4	186.0	190.0	202.4	212.7	213.6	221.1
	64	198.8	202.0	217.9	221.5	231.0	236.9	244.0
	65	177.5	190.1	180.3	190.9	198.3	205.3	203.7
	66	174.0	182.2	187.5	198.7	201.3	211.3	211.4
	67	175.0	187.4	194.0	199.8	207.4	212.1	217.0
	68	170.6	177.8	185.5	188.3	189.7	191.2	209.6
Male	69	160.6	165.9	174.1	186.2	188.9	194.0	201.1
	70	150.5	156.5	162.3	167.6	175.9	177.3	187.1
	71	179.0	184.0	192.1	197.1	207.0	213.5	208.5
	72	192.2	204.7	205.3	211.4	222.8	223.6	231.8
	73	169.4	172.6	184.9	197.7	198.6	197.5	204.4
	74	192.7	196.4	211.0	221.5	231.3	225.4	240.4
	75	159.3	168.2	172.2	174.5	188.4	188.3	196.2
	76	162.9	165.9	172.6	185.6	187.4	194.5	196.7
	77	168.3	176.2	182.3	193.1	193.8	203.5	200.9
	78	171.0	182.7	187.1	200.0	204.7	210.1	217.1
100	79	185.6	192.6	196.9	202.3	215.7	215.6	230.6
	80	170.5	178.6	190.2	192.9	204.5	199.6	210.1
	81	173.7	180.6	189.5	196.1	203.2	204.3	213.3
	82	166.8	170.8	175.0	188.7	198.3	199.4	197.8
	83	171.9	177.1	186.5	195.3	195.6	203.8	203.3
	84	161.4	169.0	176.1	181.9	185.7	190.8	190.4
	85	179.0	187.5	191.6	194.4	206.3	197.0	208.7
	86	183.8	186.8	195.8	207.3	204.0	219.7	215.8
	87	178.0	189.7	196.8	209.7	212.6	213.5	220.4
	88	167.6	176.0	183.0	189.0	196.4	194.7	204.8
300	89	198.3	208.9	213.4	225.6	233.3	237.7	245.2
	90	151.3	161.6	166.4	171.1	181.3	185.1	187.1
	91	161.7	158.6	164.3	171.4	174.0	181.1	181.7
	92	167.8	171.0	177.1	180.4	187.6	186.9	192.4
	93	167.0	175.3	176.5	184.3	196.1	198.1	209.4
	94	192.9	201.5	210.6	221.9	228.0	234.5	238.4
	95	175.9	187.9	188.8	194.8	203.0	209.4	212.6
	96	154.5	160.0	166.0	175.9	174.8	186.7	185.9

Appendix 2 -Continued
Body weights(g)

Sex	Exp. group (ng/kg/day)	Animal No.	Recovery						
			1	3	5	8	10	12	14 (days)
Vehicle control	7	297.3	310.4	319.9	335.9	347.5	356.8	367.3	
	8	288.3	295.6	305.7	315.4	327.3	336.9	347.2	
	9	358.9	372.9	380.8	397.8	411.7	421.3	430.9	
	10	361.4	376.4	385.9	402.1	415.9	423.5	440.1	
	11	292.6	303.4	315.5	331.2	341.7	360.3	364.8	
	12	321.4	335.3	343.7	359.9	366.0	374.5	383.7	
Male	31	352.1	362.8	369.5	385.4	394.1	398.6	414.0	
	32	322.2	330.4	339.4	351.6	364.7	373.1	385.0	
	33	372.3	381.0	389.2	410.0	422.1	423.8	440.1	
	34	280.0	290.9	295.1	312.0	321.3	326.4	339.0	
	35	337.1	337.3	343.4	360.2	372.0	383.7	391.3	
	36	357.5	371.3	382.5	407.0	418.0	427.4	444.4	
300	43	320.2	331.0	337.8	348.9	361.4	374.7	387.2	
	44	283.9	289.7	295.3	312.9	319.1	334.6	345.8	
	45	304.8	309.6	289.9	319.4	324.0	331.1	347.8	
	46	300.5	314.7	323.8	333.8	348.0	355.2	365.6	
	47	305.7	307.3	313.7	326.0	336.7	345.1	359.8	
	48	282.1	288.4	291.9	301.3	311.5	309.7	329.2	
Vehicle control	55	225.1	228.9	236.5	242.2	243.4	254.4	253.5	
	56	198.2	206.5	210.3	216.1	216.1	220.9	221.2	
	57	236.2	241.3	233.7	250.8	255.1	263.3	264.9	
	58	219.9	228.2	227.4	234.2	236.6	239.4	237.1	
	59	196.4	201.3	206.1	205.3	210.9	208.7	219.3	
	60	198.7	201.2	209.2	208.8	211.8	212.8	216.7	
100	79	234.0	238.8	247.2	247.6	243.1	259.4	253.2	
	80	216.0	211.6	224.3	221.8	236.4	238.7	246.3	
	81	211.9	220.0	219.9	228.6	230.1	236.2	238.7	
	82	201.3	206.7	206.5	210.2	218.2	213.4	226.0	
	83	207.7	211.4	214.0	213.2	222.9	220.5	228.7	
	84	201.1	207.4	207.5	204.4	219.9	212.9	223.4	
Female	91	186.6	191.9	192.2	197.4	195.2	203.9	205.7	
	92	195.5	202.5	200.7	207.2	208.3	215.9	214.4	
	93	210.0	212.6	219.1	225.6	227.3	238.2	236.7	
	94	237.6	240.6	243.3	253.4	255.9	257.9	265.5	
	95	213.6	221.7	222.7	228.8	227.0	233.5	238.9	
	96	188.9	193.8	195.4	193.9	201.0	202.0	209.1	

Addendum 3 28-day repeated-dose oral toxicity study in rats
Food consumption(g/rat/day)

B11-0394

Sex	Exp. group (mg/kg/day)	Animal No.	Administration							
			1	4	8	11	15	18	22	25
Vehicle control	1	17.4	18.5	19.4	19.4	20.2	18.6	19.6	19.3	18.4
	2	17.4	17.2	18.0	15.4	16.0	14.0	15.8	15.0	14.3
	3	17.6	17.4	17.4	18.1	19.8	18.7	20.2	19.1	17.7
	4	18.0	17.0	17.6	16.9	16.1	15.0	17.5	16.4	16.8
	5	15.6	15.2	16.4	17.5	16.3	14.7	17.9	16.7	14.8
	6	15.0	15.7	17.1	16.3	17.2	16.6	19.5	17.7	17.1
	7	14.6	14.4	16.0	15.9	15.7	15.6	16.6	16.0	15.6
	8	17.6	16.7	17.6	16.8	16.6	15.1	17.6	15.8	15.3
	9	18.9	19.7	21.0	21.8	21.9	19.8	20.7	19.0	19.0
	10	20.2	19.8	20.7	21.4	21.7	20.6	23.0	21.3	20.6
Halothane	11	15.4	16.5	16.1	15.8	15.8	14.9	18.0	17.0	17.0
	12	16.6	18.0	18.1	17.9	18.4	18.9	20.2	18.3	17.7
	13	18.0	15.7	18.8	17.7	18.6	18.0	18.4	16.9	16.9
	14	15.8	13.4	15.1	14.6	15.8	14.3	14.9	16.0	15.2
	15	16.7	16.9	19.7	19.6	19.6	18.3	19.4	18.2	19.4
	16	17.9	18.8	19.1	20.8	20.3	19.4	20.6	18.9	21.1
	17	18.9	16.8	19.2	21.2	21.9	19.2	20.5	18.2	19.7
	18	16.0	17.7	20.0	20.3	21.5	18.8	21.0	20.8	19.2
	19	16.5	15.8	17.2	17.3	17.3	15.7	15.8	15.5	15.1
	20	15.7	15.7	18.2	18.4	19.1	18.7	19.8	18.5	18.5
300 mg/kg/day	21	18.0	16.1	19.2	18.7	18.5	17.6	18.3	18.8	18.6
	22	18.9	18.1	20.8	21.3	20.2	18.7	20.4	20.1	20.3
	23	16.2	17.1	17.4	15.9	16.8	16.2	15.7	16.1	14.8
	24	16.4	16.7	19.4	18.4	19.6	18.3	19.4	18.8	18.2
	25	16.8	16.0	19.1	17.8	18.4	16.7	19.7	18.7	17.7
	26	16.9	16.3	20.8	21.3	20.6	18.7	19.9	20.1	20.3
	27	17.9	15.6	20.8	18.8	19.1	17.8	18.7	20.0	18.6
	28	22.0	20.7	23.0	21.7	22.8	18.6	21.0	19.7	19.4
	29	15.8	15.2	16.3	17.1	17.1	17.4	17.9	18.2	17.2
	30	16.0	15.2	17.3	17.7	19.2	17.4	19.5	18.6	17.0
100 mg/kg/day	31	17.6	17.0	20.1	20.3	20.6	20.5	20.6	19.2	18.9
	32	17.8	17.3	18.6	19.3	18.3	18.8	20.0	18.5	18.4
	33	19.2	19.5	22.7	22.1	22.9	22.2	23.5	22.4	22.7
	34	17.1	13.8	16.5	15.9	18.1	15.5	16.7	16.4	15.8
	35	15.2	14.4	17.2	17.7	18.9	19.0	19.3	19.1	18.1
	36	17.1	17.4	21.1	21.6	21.7	20.4	22.6	21.2	21.3
	37	16.7	14.9	16.6	15.3	16.0	14.7	15.4	15.9	15.4
	38	16.6	13.9	15.4	14.7	15.2	15.7	15.2	16.8	13.8
	39	18.4	14.0	18.2	17.3	17.3	15.8	16.9	15.1	14.5
	40	16.8	18.5	20.7	21.1	20.5	19.1	20.0	20.3	20.5
300 mg/kg/day	41	16.5	15.7	18.8	19.0	19.2	18.5	19.1	18.7	18.1
	42	16.5	14.4	17.1	18.4	17.9	17.5	19.0	18.9	18.3
	43	18.9	15.9	19.3	17.7	19.2	18.9	19.6	18.7	19.0
	44	16.3	14.6	17.0	17.0	16.3	15.2	16.6	15.6	16.1
	45	16.1	14.9	18.4	19.3	18.7	18.0	19.0	18.0	17.2
	46	17.7	16.9	18.8	19.0	17.5	17.3	18.4	17.6	16.8
	47	18.0	17.4	19.2	19.0	19.2	19.0	20.2	18.1	16.6
	48	16.0	15.6	19.0	16.9	19.1	18.3	19.0	19.9	17.8

Appendix 3 -Continued
Food consumption(g/rat/day)

Sex	Exp. group (mg/kg/day)	Animal No.	Administration								
			1	4	8	11	15	18	22	25	28 (days)
Vehicle control	49	15.2	14.7	14.1	12.4	12.5	11.7	13.4	11.7	11.7	12.6
	50	14.3	13.9	13.1	11.7	12.4	11.7	12.6	11.6	11.6	11.0
	51	14.0	13.2	13.3	12.5	12.7	12.5	13.4	13.3	13.3	12.2
	52	13.3	12.6	12.6	11.3	11.7	11.2	12.8	11.5	11.5	10.7
	53	15.2	13.8	12.6	12.7	12.4	11.8	13.7	14.1	14.1	13.1
	54	12.6	13.6	14.0	13.2	12.9	14.7	16.0	13.8	13.8	12.2
	55	14.5	13.8	14.6	13.8	13.1	12.9	14.3	14.4	14.4	12.5
	56	15.2	13.3	12.4	12.0	11.1	11.5	12.9	12.7	12.7	12.0
	57	14.5	14.6	15.9	15.2	13.6	14.8	18.2	15.5	15.5	13.2
	58	16.4	16.8	16.0	16.2	15.3	14.6	16.6	15.9	15.9	14.9
Female	59	13.7	14.3	13.7	13.4	13.1	13.4	13.9	12.3	12.3	12.6
	60	14.7	14.0	13.9	12.1	13.3	12.1	15.0	13.5	13.5	12.4
	61	14.3	15.1	15.4	14.7	14.6	14.1	15.5	15.8	15.8	15.9
	62	16.2	14.7	15.4	14.6	14.7	15.2	16.3	16.5	16.5	15.7
	63	14.2	13.8	14.1	12.7	12.8	12.2	12.4	12.7	12.7	14.3
	64	16.0	17.6	16.7	17.4	16.7	14.8	16.5	16.7	16.7	14.9
	65	15.4	14.0	13.4	13.4	16.1	16.1	12.6	12.5	12.5	11.7
	66	14.9	13.1	14.0	13.2	13.0	13.2	12.8	12.3	12.3	12.3
	67	15.7	16.0	14.8	13.9	13.6	15.4	13.8	12.6	12.6	15.3
	68	13.6	14.4	14.4	13.5	12.3	12.2	13.0	14.0	14.0	13.9
Male	69	11.6	12.0	12.4	12.0	11.9	11.6	12.5	11.8	11.8	12.6
	70	16.0	13.4	13.4	12.6	11.8	13.5	13.3	14.1	14.1	13.6
	71	16.9	16.7	16.2	16.8	14.9	13.4	15.9	15.5	15.5	13.9
	72	16.1	15.3	16.1	15.0	15.3	15.2	15.7	15.1	15.1	14.6
	73	14.6	11.0	13.0	12.9	11.6	10.5	11.7	10.5	10.5	12.6
	74	16.7	15.9	16.5	16.1	16.9	16.2	17.6	17.3	17.3	15.9
	75	13.6	11.6	13.4	12.3	12.3	11.5	13.0	13.3	13.3	13.3
	76	14.3	11.5	12.3	12.7	12.2	11.0	12.4	12.1	12.1	12.9
	77	14.8	14.0	14.7	14.1	13.1	13.1	14.2	12.7	12.7	13.3
	78	15.8	12.5	12.9	12.7	13.5	13.0	13.8	13.4	13.4	13.5
300	79	15.2	14.8	18.8	14.7	13.9	12.1	14.0	15.9	15.9	14.6
	80	15.4	15.5	13.9	13.0	14.2	13.9	13.8	15.1	15.1	13.1
	81	15.3	15.4	14.2	13.0	13.8	13.2	14.2	13.8	13.8	14.5
	82	13.8	11.7	14.9	13.8	14.5	11.6	13.4	13.2	13.2	12.6
	83	15.1	14.0	14.9	14.3	13.3	13.5	13.2	12.3	12.3	13.6
	84	14.9	13.7	14.0	13.1	12.9	12.7	12.7	13.2	13.2	11.9
	85	13.7	13.3	15.2	14.3	14.5	12.6	14.6	13.4	13.4	11.6
	86	15.2	13.7	15.5	16.8	14.3	13.4	14.5	13.2	13.2	14.6
	87	14.2	13.9	15.9	14.2	13.4	13.9	13.2	12.1	12.1	14.0
	88	12.7	12.7	13.6	13.2	13.3	13.7	12.8	13.1	13.1	13.8
300	89	15.5	15.1	16.1	16.7	16.2	15.0	15.8	16.0	16.0	14.5
	90	13.5	11.2	13.5	12.6	12.1	13.3	12.5	14.0	14.0	12.9
	91	12.4	10.8	12.2	11.6	10.4	10.3	11.2	10.8	10.8	11.3
	92	14.2	12.0	12.6	12.9	11.8	10.8	11.4	11.5	11.5	11.5
	93	15.7	14.4	14.9	13.6	14.3	12.4	14.7	16.1	16.1	15.6
	94	16.1	17.0	18.0	17.3	16.9	16.6	18.5	17.7	17.7	18.1
300	95	14.1	13.4	14.2	15.6	13.4	14.4	13.7	14.0	14.0	14.2
	96	13.7	11.7	13.4	12.2	11.5	11.4	12.8	11.3	11.3	12.3

Addendum 3 -Continued
Food consumption(g/rat/day)

B11-0394

Sex	Exp. group	(ng/kg/day)	Animal No.	Recovery			
				4	8	11	14 (days)
Male	Vehicle control	7	19.8	19.8	22.3	23.4	
		8	19.7	20.0	22.4	23.4	
		9	23.2	27.0	28.5	28.2	
	100	10	26.0	28.1	29.4	31.8	
		11	20.9	23.6	23.8	25.5	
		12	21.0	23.8	23.3	25.6	
Female	Vehicle control	31	21.3	24.8	26.5	28.4	
		32	19.0	22.6	24.4	24.8	
		33	24.8	27.4	27.2	29.4	
	300	34	19.1	21.4	21.6	23.7	
		35	17.6	23.1	26.0	26.8	
		36	26.0	31.2	30.9	34.6	
Male	Vehicle control	43	19.3	21.8	25.0	26.5	
		44	17.4	19.6	21.0	23.2	
		45	16.2	- a)	22.5	24.2	
	300	46	20.7	23.6	24.3	25.0	
		47	18.0	21.6	22.8	25.1	
		48	18.6	21.5	21.4	24.5	
Female	Vehicle control	55	16.5	18.4	17.6	18.5	
		56	15.7	16.9	16.9	16.3	
		57	17.7	20.1	21.1	23.5	
	300	58	18.7	19.5	17.9	19.0	
		59	16.4	17.1	18.8	18.8	
		60	17.7	18.2	17.0	19.6	
Female	Vehicle control	79	18.0	20.5	19.3	21.3	
		80	16.0	18.9	22.7	20.5	
		81	16.9	18.2	18.9	19.0	
	300	82	15.4	17.0	18.5	17.5	
		83	15.5	17.6	18.2	18.4	
		84	16.7	16.8	20.2	17.8	
Female	300	91	14.0	14.8	13.7	15.5	
		92	15.5	15.8	16.5	16.2	
		93	18.7	20.8	22.0	21.7	
	300	94	18.8	20.9	22.2	21.7	
		95	16.6	18.1	17.2	19.8	
		96	14.6	16.6	17.6	17.2	

a) : This data was missing value for incisor fracture.

Appendix 4 28-day repeated-dose oral toxicity study in rats
Hematology

B11-0394

Sex	Exp. group (mg/kg/day)	Animal No.	RBC ($\times 10^4/\text{mm}^3$)	WBC ($\times 10^2/\text{mm}^3$)	Hb (g/dl)	Ht (%)	MCV (μm^3)	MCH (pg)	MCHC (%)	Platelet ($\times 10^4/\text{mm}^3$)	Reticulio (%)	PT (sec)	APTT (sec)
	1	766	130	14.6	43.3	56.5	18.1	33.7	119.2	39	12.2	27.1	
	2	717	149	14.9	42.1	58.0	20.8	35.4	105.9	24	12.0	24.9	
	3	717	102	14.3	41.4	57.7	19.9	34.5	132.9	38	12.7	23.6	
	4	765	88	15.4	46.2	59.1	20.1	34.1	110.4	31	11.7	22.7	
	5	737	102	16.1	44.6	60.5	20.5	33.9	115.8	28	12.9	24.2	
	6	730	118	14.9	43.2	59.2	20.4	34.5	124.6	26	12.3	25.2	
Vehicle control	Recovery												
	7	834	126	15.4	44.7	53.6	18.5	34.5	121.5	33	12.8	25.5	
	8	768	114	14.7	41.5	54.0	19.1	35.4	117.6	29	12.5	36.2	
	9	761	95	16.3	43.1	56.6	20.1	35.5	133.2	26	12.1	23.4	
	10	774	122	15.0	42.8	55.3	19.4	35.0	109.0	23	12.9	28.6	
	11	765	102	15.0	41.7	54.5	19.6	36.0	111.8	12	11.4	25.0	
	12	715	124	14.8	40.9	57.2	20.7	36.2	120.2	9	11.4	21.6	
	13	669	169	13.9	39.9	59.6	20.8	34.8	124.4	36	12.5	24.7	
	14	713	90	14.3	42.2	59.2	20.1	33.9	120.3	28	12.8	23.4	
	15	716	114	13.6	40.4	56.4	19.0	33.7	127.3	31	12.1	21.7	
	16	702	93	14.4	41.9	59.7	20.5	34.4	103.8	33	12.2	24.6	
	17	746	146	14.8	42.1	56.4	19.8	35.2	122.1	26	13.0	23.5	
	18	728	98	14.2	41.3	56.7	19.5	34.4	89.8	25	12.5	24.2	
	19	773	150	15.0	58.0	19.4	33.3	104.5	27	12.4	31.0		
	20	703	113	14.3	41.7	59.3	20.3	34.3	129.6	37	12.6	26.4	
	21	736	110	13.9	41.2	56.0	18.9	33.7	107.5	20	11.9	21.0	
	22	705	108	14.4	42.1	59.7	20.4	34.2	103.6	25	11.9	21.7	
	23	770	58	14.7	42.6	55.3	19.1	34.5	95.1	25	12.2	18.9	
	24	716	78	14.3	41.4	57.8	20.0	34.5	102.9	39	12.8	29.1	
	25	821	84	15.5	45.7	55.7	18.9	33.9	136.9	27	13.9	25.9	
	26	751	127	14.9	42.9	57.1	19.8	34.7	121.7	34	12.3	24.2	
	27	720	117	15.3	45.2	62.8	21.3	33.8	103.1	33	12.5	26.5	
	28	748	70	15.6	43.8	58.6	20.9	35.6	110.1	36	11.6	25.9	
	29	727	127	14.6	43.1	59.3	20.1	33.9	109.5	38	12.2	21.3	
	30	777	94	15.3	46.1	59.3	19.7	33.2	106.5	28	13.7	25.0	
	Recovery												
	31	766	86	14.4	40.4	52.7	18.8	35.6	105.7	39	12.7	26.0	
	32	762	145	15.0	43.4	57.0	19.7	34.6	107.7	43	12.3	25.0	
	33	757	117	15.3	42.9	56.7	20.2	35.7	119.9	16	13.0	25.7	
	34	798	77	15.6	43.7	54.8	19.5	35.7	119.5	22	13.3	26.9	
	35	731	152	14.3	41.0	56.1	19.6	34.9	93.9	26	12.5	21.7	
	36	699	122	14.8	42.3	60.5	21.2	35.0	120.8	21	12.4	23.0	

Addendum 4 -Continued
Hematology

B11-0394

Sex	Exp. group (ng/kg/day)	Animal No.	RBC (x10 ⁴ /mm ³)	WBC (x10 ² /mm ³)	Hb (g/dl)	Ht (%)	MCV (μm ³)	MCH (pg)	MCHC (%)	Platelet (x10 ⁴ /mm ³)	Reticulo (%)	P T (sec)	APTT (sec)
	37	764	79	15.3	44.7	59.3	20.3	34.2	87.8	26	16.7	31.5	
	38	765	105	16.5	45.2	69.1	20.3	34.3	103.4	35	13.4	29.2	
	39	782	80	16.0	47.3	60.5	20.5	33.8	96.2	18	12.7	26.8	
	40	756	72	14.9	43.4	57.5	19.7	34.3	111.6	30	13.1	26.9	
	41	750	58	14.9	44.4	59.2	19.9	33.6	91.4	41	12.2	25.3	
	300	42	732	55	15.5	45.3	61.9	21.2	34.2	89.6	43	13.4	26.6
Male													
	43	809	101	14.5	41.9	51.8	17.9	34.6	101.4	26	13.0	28.3	
	44	737	81	14.6	41.6	56.4	19.8	35.1	114.3	34	13.2	27.2	
	45	744	105	15.2	43.1	57.9	20.4	35.3	111.5	17	12.4	22.5	
	46	831	116	15.5	43.5	52.3	18.7	35.6	121.8	13	13.3	22.8	
	47	796	158	15.5	42.9	53.9	19.5	36.1	117.1	15	12.0	25.9	
	48	778	90	15.3	42.6	54.8	19.7	35.9	105.9	13	11.1	26.1	

Addendum 4 -Continued
Hematology

B11-0394

Sex	Exp. group (mg/kg/day)	Animal No.	N-Band	Differentiation of leukocyte (%)				
				N-Seg	Eosino	Baso	Lymph	Mono
Vehicle control	1	0.0	16.0	2.5	0.0	81.5	0.0	0.0
	2	0.0	14.5	1.0	0.0	84.5	0.0	0.0
	3	0.5	20.5	1.0	0.0	78.0	0.0	0.0
	4	0.0	10.5	0.0	0.0	89.5	0.0	0.0
	5	0.0	15.0	0.0	0.0	85.0	0.0	0.0
	6	0.0	22.0	0.0	0.0	78.0	0.0	0.0
	7	0.0	12.0	0.5	0.0	86.5	1.0	0.0
	8	0.0	10.0	0.5	0.0	89.5	0.0	0.0
	9	0.0	15.5	0.5	0.0	84.0	0.0	0.0
	10	0.0	16.0	0.0	0.0	84.0	0.0	0.0
Male	11	0.0	12.5	0.5	0.0	87.0	0.0	0.0
	12	0.0	16.0	0.0	0.0	83.5	0.5	0.5
	13	0.0	21.0	1.0	0.0	78.0	0.0	0.0
	14	0.5	20.5	1.0	0.0	78.0	0.0	0.0
	15	0.5	30.0	2.0	0.0	67.5	0.0	0.0
	16	0.5	15.0	0.0	0.0	84.5	0.0	0.0
	17	0.0	30.0	0.5	0.0	69.5	0.0	0.0
	18	0.0	26.5	0.0	0.0	73.5	0.0	0.0
	19	0.0	12.5	1.5	0.0	86.0	0.0	0.0
	20	0.5	21.0	0.0	0.0	78.5	0.0	0.0
100	21	0.0	23.5	0.5	0.0	76.0	0.0	0.0
	22	0.5	23.0	0.0	0.0	76.5	0.0	0.0
	23	0.5	24.0	0.5	0.0	75.0	0.0	0.0
	24	0.5	18.5	1.5	0.0	79.5	0.0	0.0
	25	0.0	11.5	0.0	0.0	88.5	0.0	0.0
	26	0.0	11.5	0.5	0.0	88.0	0.0	0.0
	27	0.0	12.0	0.5	0.0	87.5	0.0	0.0
	28	0.5	16.5	0.0	0.0	83.0	0.0	0.0
	29	1.0	16.0	1.0	0.0	82.0	0.0	0.0
	30	1.0	14.5	0.0	0.0	84.0	0.5	0.5
Recovery	31	0.0	13.5	0.5	0.0	86.0	0.0	0.0
	32	0.0	8.0	1.0	0.0	91.0	0.0	0.0
	33	0.0	11.5	1.0	0.0	87.5	0.0	0.0
	34	0.0	12.5	1.0	0.0	86.5	0.0	0.0
	35	0.0	23.0	0.5	0.0	76.0	0.5	0.5
	36	0.0	21.0	0.5	0.0	78.5	0.0	0.0

Appendix 4 -Continued
Hematology

B11-0394

Sex	Exp. group (#S/kg/day)	Animal No.	N-Band	Differentiation of leukocyte (%)				
				N-Seg	Eosino	Baso	Lymph	Mono
	37	0.0	8.5	0.6	0.0	0.0	91.0	0.0
	38	0.0	7.0	0.0	0.0	0.0	93.0	0.0
	39	1.0	8.0	0.0	0.0	0.0	91.0	0.0
	40	0.0	13.5	0.0	0.0	0.0	86.5	0.0
	41	0.0	21.0	1.0	0.0	0.0	78.0	0.0
	42	0.0	16.5	0.5	0.0	0.0	83.0	0.0
Male	<u>Recovery</u>							
	43	0.0	12.0	1.5	0.0	0.0	86.5	0.0
	44	0.0	9.0	0.5	0.0	0.0	90.5	0.0
	45	0.0	19.0	1.0	0.0	0.0	79.5	0.5
	46	0.0	5.5	0.0	0.0	0.0	94.5	0.0
	47	0.0	11.5	0.0	0.0	0.0	88.5	0.0
	48	0.0	15.0	4.5	0.0	0.0	80.5	0.0

Addendum 4 -Continued
Hematology

B11-0394

Sex	Exp. Group (mg/kg/day)	Animal No.	RBC (x10 ⁴ /mm ³)	WBC (x10 ³ /mm ³)	Hb (g/dl)	Ht (%)	MCV (μm ³)	MCH (pg)	MCHC (%)	Platelet (x10 ⁴ /mm ³)	Reticulo (%)	PT (sec)	APTT (sec)
Male	49	734	51	14.8	41.6	56.7	20.2	35.6	128.4	18	11.4	21.2	
	50	734	62	15.2	42.7	58.2	20.7	35.6	106.0	18	12.0	19.7	
	51	727	65	14.7	41.9	57.6	20.2	35.1	136.3	20	11.4	20.3	
	52	768	70	15.7	43.7	56.9	20.4	35.9	121.1	15	11.9	20.6	
	53	727	58	14.5	41.2	56.7	19.9	35.2	132.1	22	12.3	24.7	
	54	710	69	14.4	41.2	58.0	20.3	35.0	126.6	41	11.2	23.6	
	Recovery												
	55	765	64	15.4	43.1	56.3	20.1	35.7	122.4	16	11.7	18.5	
	56	763	71	15.6	43.5	57.0	20.4	35.9	99.8	23	11.8	20.3	
	57	772	114	15.1	42.1	54.5	19.6	35.9	104.0	19	12.4	21.5	
Female	58	772	82	16.2	44.0	57.0	21.0	36.8	126.7	15	12.2	20.2	
	59	770	84	15.4	42.2	54.8	20.0	36.5	116.0	16	11.7	19.5	
	60	820	86	15.8	43.5	53.0	19.3	36.3	142.3	11	12.3	18.8	
	61	688	94	14.1	39.8	57.8	20.5	35.4	132.1	13	11.8	22.8	
	62	754	67	15.2	43.8	58.1	20.2	34.7	109.6	16	12.0	22.7	
	63	702	45	14.7	42.1	60.0	20.9	34.9	125.4	25	12.4	26.3	
	64	731	89	14.7	42.1	57.6	20.1	34.9	93.6	21	12.2	23.4	
	65	776	58	15.6	45.3	58.4	20.1	34.4	130.1	14	11.2	21.9	
	66	733	111	15.1	42.6	58.1	20.6	35.4	121.5	10	11.4	21.4	
	67	710	51	14.6	40.9	57.6	20.6	35.7	108.0	23	12.4	22.7	
30	68	748	40	15.1	43.5	58.2	20.2	34.7	111.4	18	12.4	21.3	
	69	738	67	15.1	44.0	59.6	20.5	34.3	119.8	24	12.1	21.7	
	70	759	54	15.0	42.7	56.3	19.8	35.1	114.3	26	11.8	24.6	
	71	745	104	15.5	42.9	57.6	20.8	36.1	113.8	25	11.3	17.4	
	72	729	64	15.6	46.1	63.2	21.4	33.8	115.3	27	11.3	21.4	
	73	730	61	14.5	41.7	57.1	19.9	34.8	108.6	21	12.2	25.3	
	74	695	72	14.6	40.6	58.4	21.0	36.0	111.3	23	12.2	21.9	
	75	739	65	15.0	43.6	69.0	20.3	34.4	94.7	30	12.4	21.2	
	76	773	66	15.0	43.7	56.5	19.4	34.3	110.6	29	12.1	25.6	
	77	734	82	14.8	41.3	56.3	20.2	35.8	103.3	28	12.2	20.9	
100	78	737	73	15.0	44.1	59.8	20.4	34.0	132.2	30	12.8	23.3	
	Recovery												
	79	799	86	15.6	43.8	54.8	19.5	35.6	114.4	9	12.3	19.2	
	80	759	59	15.1	42.4	55.9	19.9	35.6	127.9	15	12.3	20.4	
	81	763	67	14.7	39.7	52.0	19.3	37.0	122.8	17	12.1	15.0	
	82	723	75	14.9	41.4	57.3	20.6	36.0	130.1	12	12.2	16.3	
	83	816	61	14.9	43.4	53.2	18.3	34.3	118.2	12	12.3	17.1	
	84	747	111	15.0	41.7	55.8	20.1	36.0	112.7	15	13.0	19.9	

Addendum 4 -Continued
Hematology

Sex	Exp. Group (ug/kg/day)	Animal No.	RBC (x10 ⁶ /mm ³)	WBC (x10 ³ /mm ³)	Hb (g/dl)	Ht (%)	HCV (μm ³)	MCH (pg)	MCHC (%)	Platelet (x10 ⁴ /mm ³)	Reticulo (%)	P T (sec)	APTT (sec)
	85	693	88	15.1	4.27	61.6	21.8	35.4	116.8	20	12.3	23.2	
	86	754	89	15.6	44.5	59.0	20.7	35.1	105.9	16	12.4	26.3	
	87	701	78	14.9	42.0	59.9	21.3	35.5	126.6	33	12.2	23.7	
	88	720	92	14.7	41.9	58.2	20.4	35.1	88.5	26	11.5	15.9	
	89	749	91	15.5	43.9	58.6	20.7	35.3	102.9	37	12.1	22.1	
	90	725	97	15.4	44.4	61.2	21.2	34.7	103.0	33	12.0	18.2	
Female	<hr/>												
	Recovery												
	91	732	63	14.8	41.2	58.3	20.2	35.9	113.9	12	12.1	21.5	
	92	803	48	15.4	42.4	52.8	19.2	36.3	117.4	6	12.5	21.0	
	93	776	76	15.4	42.5	54.8	19.8	36.2	122.0	9	12.7	17.0	
	94	787	74	16.1	43.4	55.1	20.5	37.1	119.7	10	12.4	16.5	
	95	750	71	15.1	42.5	56.7	20.1	35.5	107.6	14	12.2	17.1	
	96	783	60	15.6	42.8	54.7	19.9	36.4	123.9	18	12.3	18.1	
	<hr/>												

Addendum 4 -Continued
Hematology

B11-0394

Sex	Exp. group (mg/kg/day)	Animal No.	N-Band	Differentiation of leukocyte (%)				
				N-Seg	Eosino	Baso	Lymph	Mono
Vehicle control	49	0.0	9.0	0.5	0.0	90.5	0.0	0.0
	50	1.0	20.5	0.5	0.0	78.0	0.0	0.0
	51	0.0	14.5	0.5	0.0	85.0	0.0	0.0
	52	1.0	11.5	0.0	0.0	87.5	0.0	0.0
	53	0.0	13.0	1.5	0.0	84.5	1.0	0.0
	54	0.5	30.0	0.5	0.0	69.0	0.0	0.0
	Recovery		14.0	1.0	0.0	84.5	0.5	0.5
	55	0.0	8.0	0.5	0.0	91.5	0.0	0.0
	56	0.0	14.0	1.0	0.0	85.0	0.0	0.0
	57	0.0	14.0	0.0	0.0	83.5	2.0	0.0
Female	58	0.0	14.5	0.5	0.0	96.0	0.0	0.0
	59	0.5	4.5	0.0	0.0	86.0	0.5	0.5
	60	0.0	12.0	1.5	0.0	84.5	0.0	0.0
	61	0.0	14.5	1.0	0.0	75.0	0.0	0.0
	62	0.0	24.5	0.5	0.0	82.0	0.0	0.0
	63	0.5	16.0	1.5	0.0	85.0	0.0	0.0
	64	0.0	14.5	0.5	0.0	80.5	0.5	0.5
	65	1.0	17.5	0.5	0.0	87.0	0.0	0.0
	66	0.5	12.5	0.0	0.0	83.5	0.0	0.0
	67	0.0	12.0	1.0	0.0	87.0	0.0	0.0
Male	68	0.0	16.0	0.0	0.0	84.0	0.0	0.0
	69	0.0	18.0	0.0	0.0	82.0	0.0	0.0
	70	0.5	19.0	0.0	0.0	79.5	1.0	0.0
	71	0.0	16.0	0.5	0.0	83.5	0.0	0.0
	72	0.0	12.0	0.0	0.0	88.0	0.0	0.0
	73	0.0	20.0	0.5	0.0	79.5	0.0	0.0
	74	0.5	12.5	1.5	0.0	85.5	0.0	0.0
	75	0.0	18.0	0.5	0.0	81.5	0.0	0.0
	76	0.0	19.5	0.0	0.0	80.5	0.0	0.0
	77	0.5	18.0	0.0	0.0	81.5	0.0	0.0
100	78	0.0	10.5	0.5	0.0	89.0	0.0	0.0
	Recovery		8.0	2.5	0.0	89.5	0.0	0.0
	79	0.0	16.0	1.5	0.0	82.5	0.0	0.0
	80	0.0	14.0	2.5	0.0	83.5	0.0	0.0
	81	0.0	6.5	0.0	0.0	93.0	0.0	0.0
	82	0.5	13.0	2.0	0.0	85.0	0.0	0.0
	83	0.0	11.5	1.0	0.0	86.5	1.0	0.0

Appendix 4 -Continued
Hematology

B11-0394

Sex	Exp. group (mg/kg/day)	Animal No.	Differentiation of leukocyte (%)				
			N-Seg	Rosino	Baso	Lymph	Mono
	85	0.5	22.5	0.5	0.0	76.0	0.5
	86	0.0	11.5	0.5	0.0	88.0	0.0
	87	0.0	9.5	0.5	0.0	90.0	0.0
	88	0.0	8.5	0.5	0.0	91.0	0.0
	89	0.5	18.5	1.0	0.0	80.0	0.0
	90	0.0	5.0	0.5	0.0	94.0	0.5
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Female							
	Recovery						
	91	0.0	7.0	2.0	0.0	90.5	0.5
	92	0.0	17.0	0.5	0.0	82.5	0.0
	93	0.0	10.0	0.5	0.0	89.0	0.5
	94	0.0	14.0	1.0	0.0	84.0	1.0
	95	0.0	4.0	0.5	0.0	95.5	0.0
	96	0.0	8.5	1.0	0.0	89.5	1.0

Appendix 5 28-day repeated-dose oral toxicity study in rats
Blood chemistry

Sex	Exp. group (mg/kg/day)	Animal No.	GOT (IU/l)	GPT (IU/l)	ALP (IU/l)	ChE (IU/l)	γ -GTP (IU/l)	T-Chol (mg/dl)	TG (mg/dl)	Glucose (mg/dl)	T-protein (g/dl)	Albumin (g/dl)	A/G ratio
	1	54	12	486	53	0.3	70	102	133.2	5.8	2.9	2.7	1.00
	2	59	16	409	59	1.0	62	92	129.1	6.0	2.9	2.8	0.94
	3	63	15	253	47	0.6	84	150	123.0	5.9	2.9	2.8	0.97
	4	56	16	481	29	0.7	76	95	136.8	6.8	3.0	3.0	1.07
	5	60	15	637	51	0.4	73	126	139.0	6.0	3.0	3.0	1.00
	6	68	17	447	47	0.8	76	131	120.5	6.0	2.8	2.8	0.88
	7	58	18	226	46	0.1	83	121	113.0	5.9	2.9	2.9	0.97
	8	58	15	297	105	0.3	61	101	115.3	6.0	2.8	2.8	0.88
	9	57	21	304	78	0.3	90	103	139.7	5.9	2.8	2.8	0.90
	10	70	24	363	45	0.4	71	139	120.6	6.5	3.1	3.1	0.91
	11	56	17	286	58	0.1	75	108	122.3	6.0	2.7	2.7	0.82
	12	67	22	491	69	0.0	87	79	138.0	6.1	2.7	2.7	0.79
	13	83	18	706	49	0.9	59	146	130.6	5.7	2.8	2.8	0.97
	14	66	14	560	57	0.3	90	298	129.8	5.8	2.9	2.9	1.00
	15	56	12	293	70	0.6	68	87	134.1	6.0	2.7	2.7	0.82
	16	62	13	615	44	0.5	84	107	137.0	6.0	2.9	2.9	0.94
	17	63	12	409	47	0.6	66	81	142.8	5.8	2.8	2.8	1.00
	18	54	13	487	72	0.8	79	147	145.6	5.6	2.8	2.8	1.00
	19	54	17	453	55	0.6	79	103	112.8	6.0	2.9	2.9	0.94
	20	54	14	463	54	0.3	75	111	161.4	6.0	2.8	2.8	0.88
	21	65	18	367	56	0.4	91	108	112.6	5.9	2.9	2.9	0.97
	22	54	12	517	62	0.3	71	223	165.4	5.8	2.7	2.7	0.87
	23	54	14	464	35	0.4	81	263	130.2	5.9	2.9	2.9	0.97
	24	59	14	449	62	0.2	62	125	139.8	5.8	2.8	2.8	0.93
	25	54	14	569	47	0.9	76	115	150.7	6.1	3.0	3.0	0.97
	26	58	17	519	44	0.3	87	201	167.7	6.1	3.0	3.0	0.97
	27	51	18	437	49	0.3	90	168	132.3	5.9	2.8	2.8	0.90
	28	47	20	857	53	0.4	107	393	144.3	6.0	3.0	3.0	1.00
	29	70	16	780	46	0.7	99	202	128.3	5.9	2.9	2.9	0.97
	30	62	18	513	46	0.5	67	97	184.1	6.2	3.0	3.0	0.94
	Recovery												
	31	59	18	228	59	0.1	81	92	138.0	5.9	2.7	2.7	0.84
	32	59	20	315	60	0.2	80	121	147.8	5.9	2.8	2.8	0.90
	33	68	25	230	41	0.2	55	127	122.3	6.1	2.8	2.8	0.85
	34	70	21	312	54	0.1	78	135	115.9	6.0	2.9	2.9	0.94
	35	73	18	251	73	0.3	92	111	143.4	6.1	2.8	2.8	0.85
	36	79	22	302	58	0.6	94	121	149.9	6.2	2.8	2.8	0.82

Addendum 5 -Continued
Blood chemistry

B11-0394

Sex	Exp. group (mg/kg/day)	GOT (IU/l)	GPT (IU/l)	ALP (IU/l)	ChE (IU/l)	γ -GTP (IU/l)	T-Chol (mg/dl)	TG (mg/dl)	Glucose (mg/dl)	T-protein (g/dl)	Albumin (g/dl)	A/G ratio
	37	72	28	99.8	40	0.8	109	283	111.0	5.8	2.9	1.00
	38	68	23	53.7	63	0.7	97	221	118.6	5.8	2.8	0.93
	39	59	20	58.5	47	0.4	102	221	143.4	6.1	2.9	0.91
	40	66	23	86.9	55	0.4	88	158	135.5	6.1	3.0	0.97
	41	76	35	65.5	41	0.6	131	435	85.0	5.6	2.8	1.00
	300	42	59	19	65.1	42	0.4	87	184	115.8	5.9	2.9
Male	Recovery											
	4.3	60	23	27.2	4.3	0.3	77	130	144.9	6.1	2.8	0.85
	4.4	65	24	41.9	69	0.1	72	83	139.2	5.8	2.8	0.93
	4.5	68	21	29.6	105	0.3	70	67	112.1	5.8	2.7	0.87
	4.6	78	26	26.2	50	0.3	73	109	126.9	5.7	2.7	0.90
	4.7	77	26	25.9	121	0.3	105	182	121.0	5.9	3.1	1.11
	4.8	72	24	24.9	46	0.3	74	44	118.3	6.0	2.8	0.88

Addendum 5 -Continued
Blood chemistry

B11-0394

Sex	Rsp. Group (mg/kg/day)	Animal No.	BUN (mg/dl)	Creatinine (mg/dl)	T-Bil (mg/dl)	Ca (mg/dl)	IP (ug/dl)	Na (mEq/l)	K (mEq/l)	Cl (mEq/l)
	1	8.2	0.42	0.24	9.8	7.8	143	4.2	106.4	
	2	11.2	0.48	0.26	9.6	7.5	142	4.1	104.9	
	3	9.5	0.46	0.26	9.6	6.8	143	3.9	106.2	
	4	8.6	0.50	0.23	9.8	7.1	144	4.5	108.3	
	5	8.1	0.46	0.24	10.2	7.4	142	3.9	103.3	
Vehicle control	6	9.4	0.38	0.29	9.9	7.6	142	4.4	105.3	
	Recovery									
	7	13.3	0.40	0.28	9.9	7.1	144	4.5	107.2	
	8	13.9	0.35	0.30	9.6	7.6	143	4.2	104.3	
	9	12.7	0.44	0.19	10.0	7.0	144	3.7	105.1	
	10	16.0	0.50	0.19	10.4	6.7	143	3.9	104.2	
	11	12.8	0.44	0.30	10.2	6.7	144	4.2	104.7	
	12	15.3	0.41	0.24	10.3	7.4	144	4.4	104.2	
	13	8.3	0.44	0.23	9.5	7.3	145	3.8	108.1	
	14	8.1	0.45	0.31	9.9	8.1	143	4.1	106.7	
	15	9.8	0.37	0.25	10.0	7.3	143	4.1	106.6	
	16	10.2	0.53	0.23	10.1	8.8	143	4.6	107.5	
	17	8.5	0.46	0.23	9.8	8.2	144	4.6	107.4	
	18	8.2	0.42	0.21	9.9	8.2	143	4.6	104.6	
Hale	19	7.4	0.40	0.35	9.5	7.5	141	4.3	106.6	
	20	8.1	0.50	0.20	9.8	8.4	142	3.8	105.0	
	21	8.9	0.39	0.33	10.3	8.1	143	4.2	105.0	
	22	8.4	0.51	0.21	9.8	8.4	144	4.2	107.1	
	23	7.0	0.42	0.25	10.0	8.0	144	4.4	107.0	
	24	11.0	0.45	0.22	10.0	8.9	142	4.7	104.3	
	25	7.9	0.50	0.21	9.7	7.7	144	3.7	103.8	
	26	9.6	0.54	0.23	9.8	8.4	142	3.7	100.3	
	27	7.8	0.49	0.27	10.1	8.6	144	4.1	105.3	
	28	9.0	0.45	0.28	10.6	8.4	143	4.0	105.0	
	29	6.8	0.49	0.26	10.3	8.9	144	4.4	106.5	
	30	11.2	0.61	0.22	10.6	9.1	144	3.7	107.3	
	Recovery									
	31	14.2	0.39	0.22	9.9	7.3	144	4.1	103.9	
	32	15.2	0.48	0.18	10.4	8.5	144	3.7	103.2	
	33	14.0	0.42	0.21	9.9	6.7	144	3.9	105.5	
	34	16.1	0.41	0.24	10.1	7.2	144	4.0	104.5	
	35	17.5	0.58	0.20	10.2	7.4	144	3.9	105.2	
	36	14.9	0.52	0.20	9.8	7.1	143	4.6	104.5	
100										

Appendix 5 -Continued
Blood chemistry

B11-0394

Sex	Exp. group ($\mu\text{g}/\text{kg/day}$)	Animal No.	BUN (mg/dl)	Creatinine (mg/dl)	T-Bil (mg/dl)	Ca (mg/dl)	IP (mg/dl)	Na (mEq/l)	K (mEq/l)	Cl (mEq/l)
Male	37	9.6	0.63	0.29	9.8	9.2	14.3	4.1	106.4	
	38	8.0	0.40	0.38	9.8	8.2	14.3	4.0	106.4	
	39	9.1	0.48	0.26	10.1	8.3	14.3	3.7	106.4	
	40	8.1	0.57	0.27	10.7	9.6	14.5	3.6	106.2	
	41	7.4	0.19	0.47	10.0	8.2	14.4	4.1	107.5	
	42	8.7	0.39	0.32	9.7	7.7	14.4	4.2	108.0	
	Recovery									
	43	13.0	0.63	0.19	10.2	7.6	14.5	3.7	103.1	
	44	15.9	0.42	0.25	9.8	7.6	14.4	4.0	105.0	
	45	14.6	0.37	0.26	10.0	7.5	14.5	4.3	106.1	
	46	15.3	0.48	0.24	10.8	8.7	14.4	4.4	104.7	
	47	12.5	0.36	0.25	10.4	7.2	14.6	3.8	104.6	
	48	14.2	0.39	0.27	9.8	6.4	14.3	4.2	105.5	

Addendum 5 -Continued
Blood chemistry

Sex	Exp. group (mg/kg/day)	Animal No.	GOT (IU/l)	GPT (IU/l)	ALP (IU/l)	ChE (IU/l)	γ -GTP (IU/l)	T-Chol (mg/dl)	TG (mg/dl)	Glucose (mg/dl)	T-protein (g/dl)	Albumin (g/dl)	A/G ratio
Male	49	66	15	186	0.6	66	38	121.9	5.7	2.8	0.97		
	50	59	13	35.3	4.25	0.4	47	43	107.3	6.3	3.2	1.03	
	51	56	14	245	1.96	0.8	75	34	119.5	6.2	3.1	1.00	
	52	55	12	236	3.38	0.8	78	40	107.3	6.2	3.0	0.94	
	53	59	13	196	1.77	0.6	57	35	116.7	5.9	3.0	1.03	
	Vehicle control	54	58	12	3.51	2.71	0.7	76	26	150.7	6.2	3.1	1.00
	Recovery	55	69	16	147	3.66	0.5	82	104	128.3	6.5	3.2	0.97
	56	62	16	151	1.97	0.6	86	45	116.1	6.0	3.1	1.07	
	57	74	19	197	3.28	0.8	75	68	143.2	6.5	3.0	0.86	
	58	70	21	133	5.21	0.4	71	62	114.3	6.5	3.1	0.91	
Female	59	75	13	148	3.70	0.3	68	56	117.4	6.4	3.3	1.03	
	60	104	20	205	2.69	0.7	73	35	128.5	6.7	3.4	1.03	
	61	50	13	268	2.99	0.7	78	60	132.0	6.6	3.4	1.06	
	62	59	15	473	2.58	0.6	57	34	154.3	6.4	3.2	1.00	
	63	62	14	412	2.47	0.7	61	29	128.8	6.3	3.1	0.97	
	64	44	13	144	2.76	0.7	79	34	124.0	6.2	3.0	0.94	
	65	71	15	221	2.34	0.7	58	31	125.6	6.4	3.1	0.94	
	66	63	12	231	2.58	0.8	61	36	116.8	5.8	2.9	1.00	
	67	63	14	311	1.92	0.7	66	37	124.1	6.5	3.2	0.97	
	68	61	12	371	2.14	0.9	62	62	122.9	6.1	3.1	1.03	
30	69	61	13	402	2.63	0.8	74	42	143.9	6.5	3.4	1.10	
	70	59	17	334	2.19	0.6	67	50	132.7	6.1	3.1	1.03	
	71	60	12	249	2.02	0.6	81	38	112.4	6.3	3.2	1.03	
	72	62	13	264	1.22	0.9	67	84	104.8	5.9	2.9	0.97	
	73	59	14	303	1.86	0.7	82	42	130.5	6.3	3.2	1.03	
	74	52	13	235	1.46	0.6	72	70	143.2	6.3	3.1	0.97	
	75	88	17	348	2.70	0.5	83	37	101.5	6.2	3.2	1.07	
	76	87	13	373	1.30	0.9	82	39	113.6	6.2	3.1	1.00	
	77	77	16	339	2.30	0.9	78	53	114.3	6.4	3.2	1.00	
	100	78	72	16	226	1.81	0.7	82	29	105.8	6.3	3.1	0.97
78	Recovery	79	78	23	170	2.38	0.6	102	76	117.1	6.3	2.9	0.85
	80	62	20	184	5.17	0.3	71	44	125.1	6.2	3.0	0.94	
	81	81	22	195	3.62	0.5	92	46	126.9	6.3	3.1	0.97	
	82	95	18	139	1.82	0.6	75	46	114.3	6.2	3.0	0.94	
	83	67	18	180	2.45	0.3	75	35	139.3	6.2	3.0	0.94	
	84	74	15	128	3.45	0.5	78	49	127.8	6.2	3.0	0.94	

Addendum 5 -Continued
Blood chemistry

B11-0394

Sex	Exp. group (mg/kg/day)	Animal No.	GOT (IU/l)	GPT (IU/l)	ALP (IU/l)	ChE (IU/l)	γ -GTP (IU/l)	T-Chol (mg/dl)	TG (mg/dl)	Glucose (mg/dl)	T-protein (g/dl)	Albumin (g/dl)	A/G ratio
			85	91	13	392	132	0.7	110	65	113.4	6.4	3.3
	86	97	13	581	130	0.8	128	123	86.1	6.2	3.2	1.07	
	87	87	11	349	123	0.9	112	67	116.1	6.2	3.2	1.07	
	88	82	14	433	112	0.7	116	212	96.9	6.1	3.1	1.03	
	89	93	14	297	109	1.1	99	158	113.1	6.0	2.9	0.94	
	90	90	17	318	142	0.9	104	39	96.3	6.4	3.2	1.00	
<hr/>													
Female													
	91	66	13	173	267	0.6	99	40	132.9	6.7	3.2	0.91	
	92	73	17	139	447	0.7	102	43	109.9	6.3	3.1	0.97	
	93	80	17	178	294	0.8	86	66	128.6	6.6	3.0	0.83	
	94	97	18	242	268	0.3	82	47	124.3	6.6	3.2	0.94	
	95	66	12	217	429	0.3	82	32	141.7	6.6	3.1	0.89	
	96	80	19	340	311	0.4	68	61	125.1	6.3	3.1	0.97	

Addendum 5 -Continued
Blood chemistry

BII-0394

Sex	Exp. group (mg/kg/day)	Animal No.	BUN (mg/dl)	Creatinine (mg/dl)	T-Bili (mg/dl)	Ca (mg/dl)	IP (mg/dl)	Na (mEq/l)	K (mEq/l)	C1 (mEq/l)
Male	49	11.1	0.50	0.20	9.8	7.1	142	4.3	104.2	
	50	15.8	0.61	0.22	9.8	6.1	141	4.3	109.4	
	51	11.6	0.47	0.22	10.1	6.7	142	4.3	108.5	
	52	11.8	0.48	0.21	10.2	7.2	143	3.8	106.9	
	53	10.0	0.52	0.20	10.0	7.8	142	4.4	108.4	
	54	8.8	0.49	0.21	10.1	7.1	142	4.0	107.5	
	Recovery									
	55	16.2	0.60	0.22	9.9	5.5	143	4.8	110.7	
	56	18.1	0.56	0.21	9.6	5.8	143	4.7	110.3	
	57	16.8	0.62	0.15	9.7	6.1	143	3.3	107.3	
Female	58	14.4	0.46	0.22	10.3	6.6	144	4.0	107.3	
	59	14.0	0.50	0.23	9.7	5.5	144	4.5	108.8	
	60	15.7	0.57	0.08	9.9	6.5	144	4.4	107.2	
	61	10.6	0.33	0.34	10.4	6.3	143	4.1	106.2	
	62	11.7	0.63	0.18	9.9	7.6	143	3.1	106.9	
	63	8.6	0.47	0.22	10.2	7.7	142	4.2	108.2	
	64	11.0	0.43	0.22	10.3	7.9	142	4.3	106.3	
	65	12.2	0.56	0.22	9.8	7.5	143	4.1	107.9	
	66	10.2	0.47	0.24	10.0	8.5	140	4.8	105.8	
	67	9.2	0.47	0.24	10.1	6.9	144	4.1	108.4	
Male	68	9.3	0.51	0.23	9.7	7.6	144	4.0	111.9	
	69	12.0	0.45	0.28	10.4	7.7	142	4.0	106.3	
	70	10.3	0.45	0.24	9.8	6.3	142	3.9	109.4	
	71	10.9	0.48	0.23	10.4	7.9	142	3.8	106.4	
	72	9.1	0.51	0.22	10.1	8.7	145	3.7	108.0	
	73	10.8	0.51	0.25	10.2	7.6	142	4.1	106.7	
	74	10.4	0.45	0.22	10.2	7.1	141	4.3	106.1	
	75	11.8	0.51	0.26	9.9	7.5	142	4.1	106.8	
	76	11.1	0.47	0.25	9.7	7.4	144	4.4	110.3	
	77	13.2	0.43	0.23	10.3	7.8	141	4.2	106.2	
Female	78	12.9	0.48	0.26	10.0	7.5	141	4.7	108.9	
	Recovery									
	79	19.5	0.59	0.17	10.3	6.8	145	3.9	106.9	
	80	20.0	0.54	0.26	10.0	6.1	144	4.1	107.0	
	81	18.0	0.57	0.18	10.2	6.8	143	4.7	106.6	
	82	15.7	0.41	0.22	9.8	6.7	143	4.6	106.6	
	83	13.6	0.46	0.25	9.8	6.3	143	4.3	108.2	
	84	18.3	0.55	0.20	9.7	5.9	144	4.0	109.1	

Addendum 5 -Continued
Blood chemistry

B11-0394

Sex	Exp. group (mg/kg/day)	BUN (mg/dl)	Creatinine (mg/dl)	T-Bil (mg/dl)	Ca (mg/dl)	IP (mg/dl)	Na (mEq/l)	K (mEq/l)	Cl (mEq/l)
	85	8.8	0.46	0.24	9.8	8.0	14.3	4.5	108.1
	86	12.1	0.49	0.23	10.0	7.9	14.2	4.2	106.9
	87	10.6	0.45	0.25	9.9	8.8	14.4	4.1	106.2
	88	9.9	0.40	0.27	10.1	8.3	14.3	4.4	106.7
	89	10.8	0.42	0.24	9.9	8.4	14.2	4.4	107.4
	90	12.5	0.42	0.26	10.2	7.7	14.4	3.8	108.2
<hr/>									
Female									
	91	15.9	0.58	0.16	10.2	6.2	14.3	4.5	108.5
	92	17.5	0.49	0.18	9.5	5.8	14.2	4.2	107.2
	93	14.8	0.55	0.15	10.0	6.3	14.2	4.0	106.0
	94	18.9	0.69	0.19	10.1	6.9	14.4	4.0	106.9
	95	15.9	0.51	0.24	10.3	6.4	14.5	4.2	108.5
	96	16.2	0.55	0.22	10.0	7.4	14.5	4.0	107.4

Addendum 6 28-day repeated-dose oral toxicity study in rats
Urinalysis

Sex	Exp. group (mg/kg/day)	Animal No.	Volume (ml)	Color	pH	Protein	Ketones	Bilirubin	Occult Blood	Glucose	Urobilinogen (EU/dl)
	1	17	SY	6.5	±	±	-	-	-	-	0.1
	2	5	Y	6.0	++	++	-	-	-	-	0.1
	3	12	SY	6.5	+	++	-	-	-	-	0.1
	4	7	Y	6.5	+	++	-	-	-	-	0.1
	5	4	YB	5.0	++	++	-	-	-	-	0.1
Vehicle	6	6	Y	6.5	+	±	-	-	-	-	0.1
control	Recovery										
	7	17	SY	7.0	±	±	-	-	-	-	0.1
	8	8	Y	6.5	+	++	-	-	-	-	0.1
	9	19	SY	7.0	±	++	-	-	-	-	0.1
	10	7	Y	7.0	++	++	-	-	-	-	0.1
	11	17	SY	6.5	±	++	-	-	-	-	0.1
	12	5	Y	6.5	++	±	-	-	-	-	0.1
	13	5	Y	6.0	++	++	-	-	-	-	0.1
	14	13	SY	6.5	+	++	-	-	-	-	0.1
10	15	7	Y	6.5	+	++	-	-	-	-	0.1
	16	7	Y	6.5	+	++	-	-	-	-	0.1
	17	10	Y	6.5	+	++	-	-	-	-	0.1
	18	14	SY	6.5	+	++	-	-	-	-	0.1
	19	19	SY	6.5	±	++	-	-	-	-	0.1
	20	15	SY	6.5	±	++	-	-	-	-	0.1
	21	5	Y	5.0	++	++	-	-	-	-	0.1
	22	7	Y	6.0	+	++	-	-	-	-	0.1
	23	9	Y	6.0	+	++	-	-	-	-	0.1
	24	3	YB	5.0	++	±	-	-	-	-	0.1
	25	13	SY	6.5	±	++	-	-	-	-	0.1
	26	11	Y	6.5	+	++	-	-	-	-	0.1
	27	24	SY	6.5	+	++	-	-	-	-	0.1
	28	7	Y	6.0	+	++	-	-	-	-	0.1
	29	15	SY	6.5	±	++	-	-	-	-	0.1
100	30	4	Y	6.0	++	±	-	-	-	-	1
	Recovery										
	31	15	SY	6.5	±	++	-	-	-	-	0.1
	32	8	Y	7.0	+	++	-	-	-	-	0.1
	33	21	SY	6.5	±	++	-	-	-	-	0.1
	34	5	Y	6.5	++	++	-	-	-	-	1
	35	4	Y	6.0	++	++	-	-	-	-	1
	36	16	SY	6.5	±	++	-	-	-	-	0.1

SV : Slightly yellow
Y : Yellow

Addendum 6 -Continued
Urinalysis

B11-0394

	Sex	Exp.-group (mg/kg/day)	Animal No.	Volume (ml)	Color	pH	Protein	Ketones	Bilirubin	Occult Blood	Glucose	Urobilinogen (EU/dl)
		37	20	SY	6.5	+	±	-	-	-	-	0.1
		38	18	SY	6.5	+	±	-	-	±	-	0.1
		39	19	SY	6.5	+	+	-	-	-	-	0.1
		40	28	SY	6.5	+	+	-	-	-	-	0.1
		41	24	SY	6.0	+	+	-	-	-	-	0.1
	Male	300	42	4	Y	5.0	++	+	-	-	-	0.1
			Recovery									
		43	19	SY	7.0	+	±	-	-	-	-	0.1
		44	8	Y	6.5	+	±	-	-	-	-	0.1
		45	15	SY	6.5	+	+	-	-	-	-	0.1
		46	15	SY	6.5	+	+	-	-	-	-	0.1
		47	18	SY	6.5	+	+	-	-	-	-	0.1
		48	12	Y	7.0	+	+	-	-	-	-	0.1

SY : Slightly yellow
Y : Yellow

Addendum 6 -Continued
Urinalysis

BII-0394

Sex	Exp. group (mg/kg/day)	Animal No.	Volume (ml)	Color	pH	Protein	Ketones	Bilirubin	Occult Blood	Glucose	Urobilinogen (EU/dl)
Vehicle control	49	3	Y	6.0	++	-	-	-	-	-	0.1
	50	6	Y	6.5	+	-	-	-	-	-	0.1
	51	13	SY	6.5	±	-	-	-	-	-	0.1
	52	10	Y	6.5	±	-	-	-	-	-	0.1
	53	10	Y	6.5	±	-	-	-	-	-	0.1
	54	11	SY	6.5	±	-	-	-	-	-	0.1
	Recovery										
	55	24	SY	6.5	-	-	-	-	-	-	0.1
	56	13	SY	6.5	±	-	-	-	-	-	0.1
	57	5	Y	7.0	+	-	-	-	-	-	0.1
Female	58	22	SY	6.5	-	-	-	-	-	-	0.1
	59	20	SY	7.0	±	-	-	-	-	-	0.1
	60	13	SY	7.0	±	-	-	-	-	-	0.1
	61	11	SY	6.5	±	-	-	-	-	-	0.1
	62	5	Y	6.5	+	-	-	-	-	-	0.1
	63	10	SY	6.5	±	-	-	-	-	-	0.1
	64	10	SY	6.5	±	-	-	-	-	-	0.1
	65	8	Y	6.5	±	-	-	-	-	-	0.1
	66	14	SY	6.5	±	-	-	-	-	-	0.1
	67	9	Y	6.5	±	-	-	-	-	-	0.1
30	68	15	SY	6.5	±	-	-	-	-	-	0.1
	69	13	SY	6.5	±	-	-	-	-	-	0.1
	70	6	Y	6.0	+	-	-	-	-	-	0.1
	71	6	Y	6.0	+	-	-	-	-	-	0.1
	72	10	Y	6.5	±	-	-	-	-	-	0.1
	73	10	Y	6.5	±	-	-	-	-	-	0.1
	74	12	SY	6.5	±	-	-	-	-	-	0.1
	75	16	SY	6.5	±	-	-	-	-	-	0.1
	76	10	SY	6.5	±	-	-	-	-	-	0.1
	77	6	Y	6.0	+	-	-	-	-	-	0.1
100	78	17	SY	6.5	±	-	-	-	-	-	0.1
	Recovery										
	79	7	Y	6.5	±	-	-	-	-	-	0.1
	80	23	SY	6.5	-	-	-	-	-	-	0.1
SY : Slightly yellow Y : Yellow											

Addendum 6 -Continued
Urinalysis

B11-0394

Sex	Exp. group (mg/kg/day)	Animal No.	Volume (ml)	Color	pH	Protein	Ketones	Bilirubin	Occult Blood	Glucose	Urobilinogen (EU/dl)
	85	24	SY	6.5	-	-	-	-	-	-	0.1
	86	13	SY	6.0	±	-	-	-	-	-	0.1
	87	20	SY	6.0	+	-	-	-	-	-	0.1
	88	15	SY	6.5	+	-	-	-	-	-	0.1
	89	12	SY	6.5	+	-	-	-	-	-	0.1
Female	300	90	20	SY	6.5	+	-	-	-	-	0.1
<hr/>											
Recovery											
	91	12	SY	7.0	+	-	-	-	-	-	0.1
	92	9	Y	7.0	+	-	-	-	-	-	0.1
	93	11	SY	6.5	+	-	-	-	-	-	0.1
	94	14	SY	6.5	+	-	-	-	-	-	0.1
	95	23	SY	7.0	-	-	-	-	-	-	0.1
	96	17	SY	6.5	+	-	-	-	-	-	0.1

SY : Slightly yellow

Y : Yellow

Addendum 7 28-day repeated-dose oral toxicity study in rats
Absolute organ weights

BII-0394

Sex	Rin group (ng/kg/day)	Animal No.	Spleen (g)	Liver (g)	Kidney (g)	Brain (g)	Testis (g)	Adrenal gland (mg)	Ovary (mg)	Body weight (g)
Vehicle control	1	0.52	10.03	2.27	1.83	2.40	48.2	-	319.5	
	2	0.54	8.22	1.87	1.91	2.60	39.8	-	271.6	
	3	0.51	9.09	2.04	1.88	2.63	38.0	-	300.0	
	4	0.45	8.73	1.85	1.79	2.51	39.4	-	271.5	
	5	0.54	8.18	1.86	2.07	2.29	32.0	-	281.5	
	6	0.57	8.27	2.26	1.70	2.61	35.6	-	278.2	
	7	0.70	10.05	2.36	1.93	2.85	45.0	-	344.0	
	8	0.72	8.86	2.10	1.93	2.91	43.3	-	322.5	
	9	0.77	12.00	2.90	1.99	3.24	52.5	-	403.5	
	10	0.75	11.83	2.75	1.98	3.35	63.1	-	411.4	
Male	11	0.47	9.10	2.20	1.98	2.83	43.4	-	341.2	
	12	0.67	12.08	2.69	2.06	3.41	52.6	-	361.3	
	13	0.59	10.04	2.37	1.99	2.99	40.4	-	301.1	
	14	0.45	7.93	1.71	2.00	2.52	34.3	-	267.8	
	15	0.54	12.15	2.28	1.98	3.09	45.1	-	323.2	
	16	0.56	10.49	2.10	2.03	3.07	41.6	-	325.6	
	17	0.58	9.94	2.43	2.01	2.85	43.0	-	327.2	
	18	0.57	10.19	2.58	2.19	2.84	38.9	-	319.6	
	19	0.53	8.12	1.87	1.88	2.88	45.3	-	265.3	
	20	0.54	10.35	2.20	1.86	2.91	45.5	-	310.7	
Female	21	0.69	10.20	2.53	1.99	2.84	31.1	-	299.1	
	22	0.58	12.13	2.52	1.95	2.82	53.0	-	331.1	
	23	0.39	9.09	2.00	1.83	2.87	48.6	-	267.5	
	24	0.58	10.69	2.38	2.02	2.65	45.1	-	315.1	
	25	0.46	10.09	2.25	1.89	2.42	49.6	-	299.6	
	26	0.55	12.82	2.38	1.83	3.09	46.4	-	331.3	
	27	0.66	11.51	2.38	1.95	2.96	53.3	-	307.0	
	28	0.42	12.67	2.19	1.90	2.67	53.2	-	322.0	
	29	0.52	9.94	2.09	1.87	2.60	32.7	-	285.1	
	30	0.43	10.77	1.90	1.87	2.74	40.6	-	289.8	
	31	0.70	10.81	2.76	1.93	2.82	52.9	-	391.0	
	32	0.67	10.90	2.36	1.96	3.02	49.9	-	358.5	
	33	0.63	11.34	2.75	1.96	3.06	55.0	-	414.9	
	34	0.66	8.94	2.46	1.93	3.10	41.5	-	317.8	
	35	0.71	11.19	2.43	1.87	2.12	10.7	-	370.0	
	36	0.77	12.99	2.72	1.91	3.05	58.2	-	414.1	
	Recovery									

Addendum 7 -Continued
Absolute organ weights

B11-0394

Sex	Exp. group (ng/kg/day)	Animal No.	Spleen (g)	Liver (g)	Kidney (g)	Brain (g)	Testis (g)	Adrenal gland (mg)	Ovary (mg)	Body weight (g)
	37	0.40	9.78	1.83	1.77	2.87	39.8	-	245.9	
	38	0.33	9.77	1.88	1.72	2.57	34.9	-	233.0	
	39	0.39	11.48	2.01	1.89	2.46	39.6	-	269.0	
	40	0.44	13.68	2.43	1.91	2.63	47.6	-	302.5	
	41	0.46	12.30	2.18	1.80	3.08	43.6	-	275.6	
	42	0.33	11.33	2.14	1.85	2.70	47.6	-	269.8	
Male	<u>Recovery</u>									
	4.3	0.59	11.93	2.33	1.82	2.76	30.9	-	358.9	
	4.4	0.50	9.33	2.22	1.96	3.04	41.1	-	323.7	
	4.5	0.48	9.07	2.39	1.98	3.08	48.2	-	323.8	
	4.6	0.73	10.21	2.47	1.95	2.86	38.9	-	343.7	
	4.7	0.71	10.31	2.55	1.96	2.88	59.8	-	335.1	
	4.8	0.48	8.18	2.19	1.97	3.00	55.8	-	299.1	

Appendix 7 -Continued
Absolute organ weights

B11-0394

Sex	Exp. group (mg/kg/day)	Animal No.	Spleen (g)	Liver (g)	Kidney (g)	Brain (g)	Testis (g)	Adrenal gland (mg)	Ovary (mg)	Body weight (g)
Male	49	0.34	6.43	1.37	1.90	-	-	52.8	79.7	200.8
	50	0.29	4.95	1.34	1.83	-	-	42.0	73.0	180.4
	51	0.35	5.95	1.34	1.78	-	-	44.4	65.9	201.6
	52	0.37	5.63	1.26	1.75	-	-	47.4	80.0	188.1
	53	0.34	5.53	1.22	1.76	-	-	43.7	86.2	185.5
	54	0.43	6.23	1.43	1.84	-	-	55.6	60.9	199.2
Vehicle control	Recovery	55	0.48	6.33	1.32	1.78	-	53.0	69.2	240.7
	56	0.43	5.52	1.38	1.81	-	-	44.3	80.3	208.7
	57	0.51	6.99	1.65	1.95	-	-	57.8	103.5	244.8
	58	0.52	6.32	1.48	1.83	-	-	77.5	82.3	223.8
	59	0.53	5.99	1.56	1.75	-	-	52.9	107.8	200.4
	60	0.45	5.90	1.53	1.76	-	-	63.5	112.5	200.9
	61	0.50	7.42	1.56	1.77	-	-	51.7	78.2	222.6
	62	0.52	7.30	1.49	1.71	-	-	56.8	70.2	214.0
	63	0.43	6.68	1.46	1.89	-	-	56.1	78.5	208.2
	64	0.54	7.48	1.75	1.89	-	-	45.4	64.1	233.6
Female	65	0.32	6.40	1.39	1.72	-	-	60.1	81.8	195.7
	66	0.42	5.80	1.37	1.77	-	-	53.5	80.7	199.2
	67	0.34	6.91	1.57	1.85	-	-	62.7	76.1	207.9
	68	0.31	6.49	1.59	1.87	-	-	56.4	74.3	200.2
	69	0.43	6.24	1.36	1.69	-	-	27.1	68.2	188.1
	70	0.28	6.04	1.39	1.68	-	-	42.1	67.7	178.6
	71	0.36	6.86	1.65	1.80	-	-	50.7	76.2	202.3
	72	0.32	7.16	1.62	1.84	-	-	57.7	104.2	218.9
	73	0.32	6.78	1.40	1.85	-	-	48.3	81.3	194.2
	74	0.43	8.25	1.78	1.79	-	-	56.7	77.3	228.4
100	75	0.38	5.75	1.30	1.69	-	-	40.0	79.9	184.2
	76	0.25	6.09	1.20	1.70	-	-	41.6	75.0	184.6
	77	0.32	6.57	1.54	1.72	-	-	46.1	99.6	192.5
	78	0.40	6.54	1.61	1.72	-	-	52.2	82.7	206.9
	79	0.54	7.15	1.53	1.91	-	-	78.3	90.2	238.5
	80	0.36	6.35	1.48	1.78	-	-	53.3	82.4	228.9
81	81	0.36	6.36	1.56	1.76	-	-	52.1	79.4	223.2
	82	0.47	5.82	1.64	1.82	-	-	50.2	73.8	205.5
	83	0.37	5.83	1.70	1.78	-	-	70.6	93.8	208.6
	84	0.50	6.01	1.42	1.79	-	-	61.3	92.2	204.2

Addendum 9 - Continued
Pathological findings

B11-0394

Sex	Exp. group (mg/kg/day)	Animal No.	Fate	Gross findings	Histopathological findings
Male	300	47	ta	Oral cavity Whitish region of incisor (multiple)	Liver Microvesicular steatosis of hepatocytes† Incisor Degeneration and irregular alignment of ameloblasts at stage of maturation†
		48	ta	Oral cavity Whitish region of incisor (multiple)	Incisor Degeneration and irregular alignment of ameloblasts at stage of maturation†

Addendum 9 - Continued
Pathological findings

B11-0394

Sex	Exp. group (mg/kg/day)	Animal No.	Fate	Gross findings	Histopathological findings
		49	ta	NAD	NAD
		50	ta	NAD	Kidney Mineralization in corticomedullary junction+
		51	ta	NAD	NAD
		52	ta	NAD	NAD
		53	ta	NAD	NAD
		54	ta	NAD	NAD
Vehicle control					
	Recovery group				
		55	ta	NAD	NAD
		56	ta	NAD	NAD
		57	ta	NAD	NAD
		58	ta	NAD	NAD
		59	ta	NAD	NAD
		60	ta	NAD	NAD
Female					
		61	ta	Glandular stomach Blackish region of mucosa (spotty)	Glandular stomach Necrosis of mucosat
		62	ta	NAD	Not examined
		63	ta	NAD	Not examined
10		64	ta	NAD	Not examined
		65	ta	NAD	Not examined
		66	ta	NAD	Not examined
		67	ta	NAD	Not examined
		68	ta	NAD	Not examined
		69	ta	NAD	Not examined
		70	ta	NAD	Not examined
30		71	ta	NAD	Not examined
		72	ta	NAD	Not examined

Addendum 9 - Continued
Pathological findings

B11-0394

Sex	Exp. group (mg/kg/day)	Animal No.	Fate	Gross findings	Histopathological findings
		73	ta	NAD	NAD
		74	ta	NAD	NAD
		75	ta	NAD	NAD
		76	ta	NAD	NAD
		77	ta	NAD	NAD
		78	ta	NAD	NAD
100					
		Recovery group			
		79	ta	NAD	NAD
		80	ta	NAD	NAD
		81	ta	NAD	NAD
		82	ta	NAD	NAD
		83	ta	NAD	NAD
		84	ta	NAD	NAD
Female					
		85	ta	Liver Enlargement Glandular stomach Blackish region of mucosa (spotty)	Liver Ground glass appearance of hepatocytes+ Swelling of hepatocytes+ Glandular stomach Necrosis of mucosat
		86	ta	NAD	Liver Swelling of hepatocytes+
		87	ta	Liver Enlargement	Liver Ground glass appearance of hepatocytes+ Swelling of hepatocytes+
300					
		88	ta	Liver Enlargement	Liver Ground glass appearance of hepatocytes+ Swelling of hepatocytes+
		89	ta	NAD	Liver Swelling of hepatocytes+
		90	ta	Liver Enlargement	Liver Ground glass appearance of hepatocytes+ Swelling of hepatocytes+

Addendum 9 - Continued
Pathological findings

B11-0394

Sex	Exp. group (mg/kg/day)	Animal No.	Fate	Gross findings	Histopathological findings
Recovery group					
		91	ta	Oral cavity Whitish region of incisor (multiple)	Incisor Degeneration and irregular alignment of ameloblasts at stage of maturation†
		92	ta	Oral cavity Whitish region of incisor (multiple)	Incisor Degeneration and irregular alignment of ameloblasts at stage of maturation†
		93	ta	Oral cavity Whitish region of incisor (multiple)	Incisor Degeneration and irregular alignment of ameloblasts at stage of maturation†
Female	300				
		94	ta	Oral cavity Whitish region of incisor (multiple)	Incisor Degeneration and irregular alignment of ameloblasts at stage of maturation†
		95	ta	Oral cavity Whitish region of incisor (multiple)	Incisor Degeneration and irregular alignment of ameloblasts at stage of maturation†
		96	ta	Oral cavity Whitish region of incisor (multiple)	Incisor Degeneration and irregular alignment of ameloblasts at stage of maturation†

APPENDIX

PHYSICOCHEMICAL REPORT

Person in Charge of Chemical Analysis:

Signed in original

March 18, 1997

Sponsor:

Testing Facilities:

Test Substance: BPFB

- Tests:
- 1) Stability Test of the Test Substance
 - 2) Stability Test of the Test Substance in the Dosing Formulations

- Date of the Test:
- 1) October 2, December 4, 1996
 - 2) October 14, 17, 21, 1996

- Methods:
- 1) Stability Test of the Test Substance
The test substance was taken ($n=1$) before and after dosing, and analyzed qualitatively with infrared spectrophotometer (IR-470, Shimadzu). The test substance was stored at a room temperature during the test period.
 - 2) Stability Test of the Test Substance in the Dosing Formulations
Middle layer of the dosing formulation was taken ($n=3$) just after preparation, in the middle and 7 days after. After pre-treatment, the concentration of the test substance was analyzed with gas chromatography (GC). The dosing formulations were stored in a cold and dark place during the test period.

- Results:
- 1) Stability Test of the Test Substance (Figs. 1, 2, 3)
IR spectrum of the test substance was identical with IR spectrum provided from the sponsor. There were no differences of IR spectrum pattern between before and after dosing periods. Therefore, the test substance was stable during the test period.
 - 2) Stability Test of the Test Substance in Dosing Formulations (Table 1)
The concentration of the test substance in dosing formulations was in a range $\pm 10\%$ of the specified concentration after 7 days, and the test substance was stable.

1. Pre-treatment of the Dosing Formulation

1) 10 w/v% Dosing Formulation

Accurate 0.5 ml of the dosing formulation was diluted with acetone to make 50 ml. Accurate 1 ml of this solution was diluted with acetone to make 20 ml and served for GC.

The dilution rate of the sample was 2,000.

2) 0.05 w/v% Dosing Formulation

Accurate 1 ml of the dosing formulation was diluted with acetone to make 10 ml and served for GC.

The dilution rate of the sample was 10.

2. Preparation of the Standard solution

Accurate 0.1 g (0.1030 g) of the test substance was weighed and dissolved with acetone to make 10 ml. Accurate 1 ml of this solution was diluted with acetone to make 10 ml (undiluted standard solution: 1,030 µg/ml). Accurate 1 ml of this solution was diluted with acetone to make 20 ml (standard solution: 51.5 µg/ml).

3. Analytical Conditions

Instrument:	Gas chromatography (GC-9A, Shimadzu)
Column:	G-column (G-250 F.T. 1µm) 1.2 mm I.D. × 20 m
Temperature:	60°C
Gas:	Helium
Flow rate:	20 ml/min
Detector:	FID
Detector temperature:	300°C
Recorder:	Chromatopac (C-R7A, Shimadzu)
Injected amount:	1 µl

Under the analytical conditions mentioned above, the calibration curve was the straight line which passed through the origin of the coordinates with a correlation coefficient of 0.999 (Fig. 4).

A typical calibration curve are shown in Fig. 5.

4. Calculation

The test substance in each sample was measured by GC assay to calculate concentration of the test substance in each dosing formulation (C: w/v%) using the following equation:

$$C = \frac{Cs \times A \times D}{As \times 10,000}$$

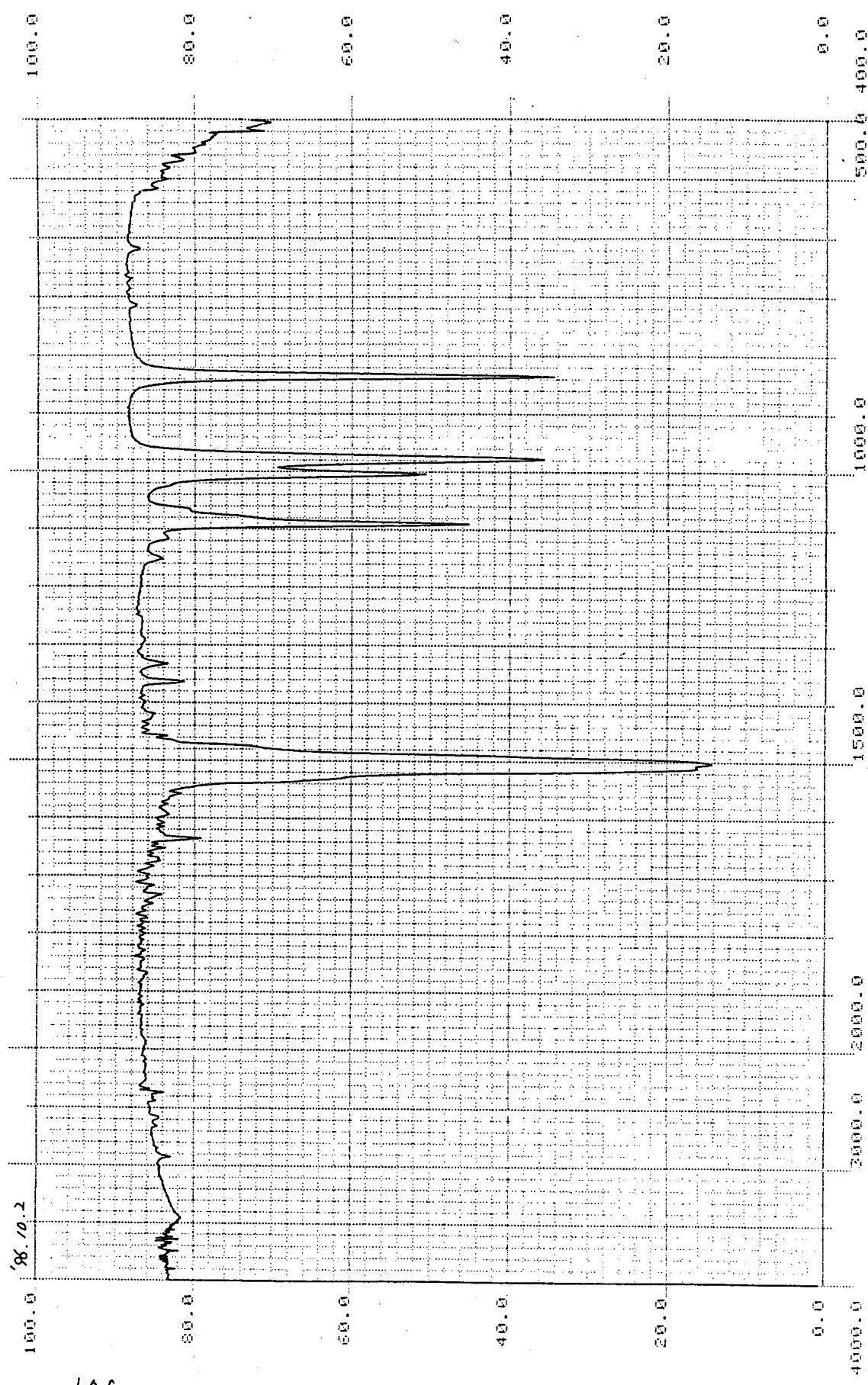
where:

Cs: Concentration of the test substance in the standard solution ($\mu\text{g/ml}$)

As: Peak area of the test substance in the standard solution

A: Peak area of the test substance in each GC sample

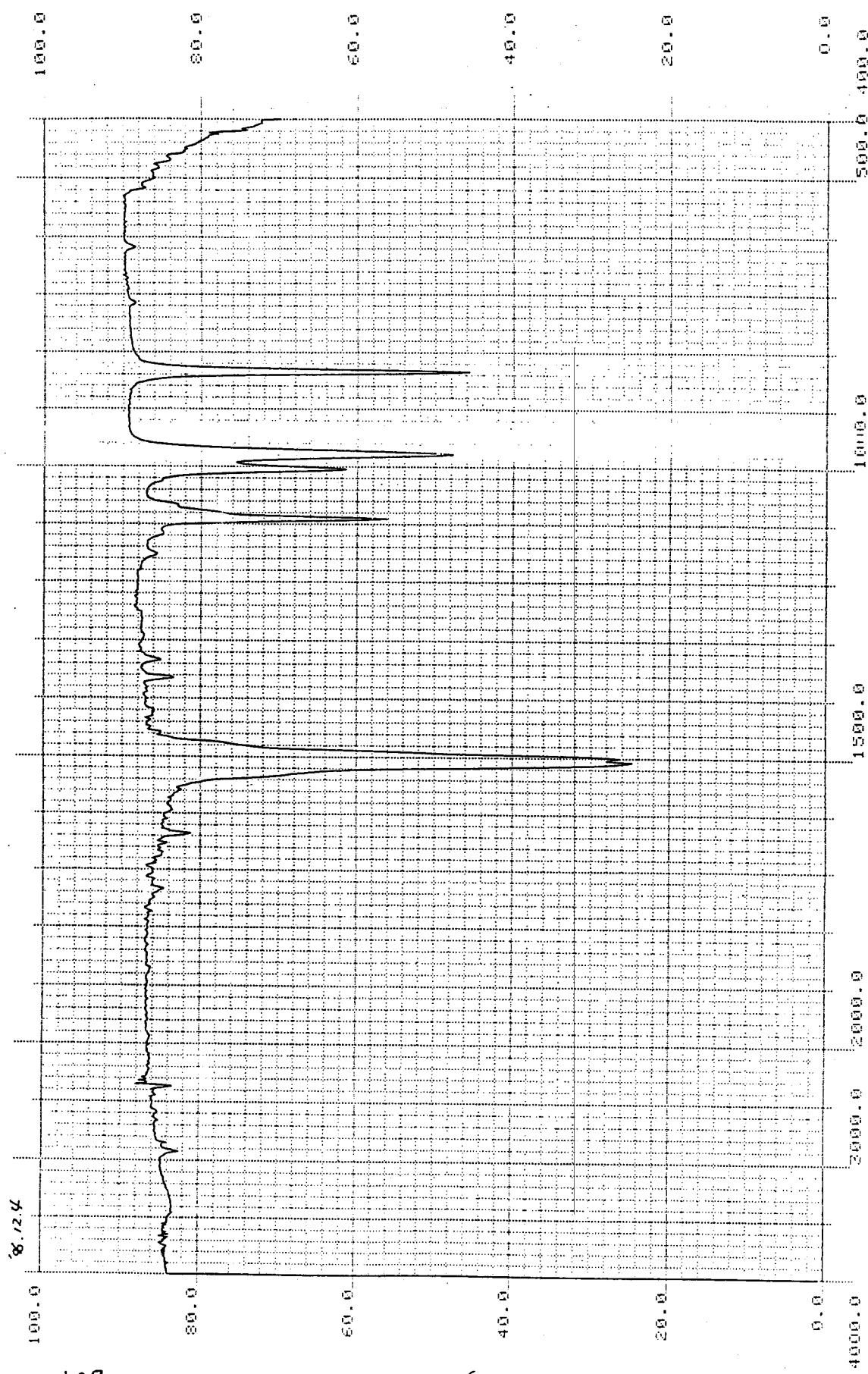
D: Dilution rate of each GC sample



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Fig. 1 IR spectrum measured prior to the administration period

B11-0394



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Fig.2 IR spectrum measured after the end of the administration period

B11-0394

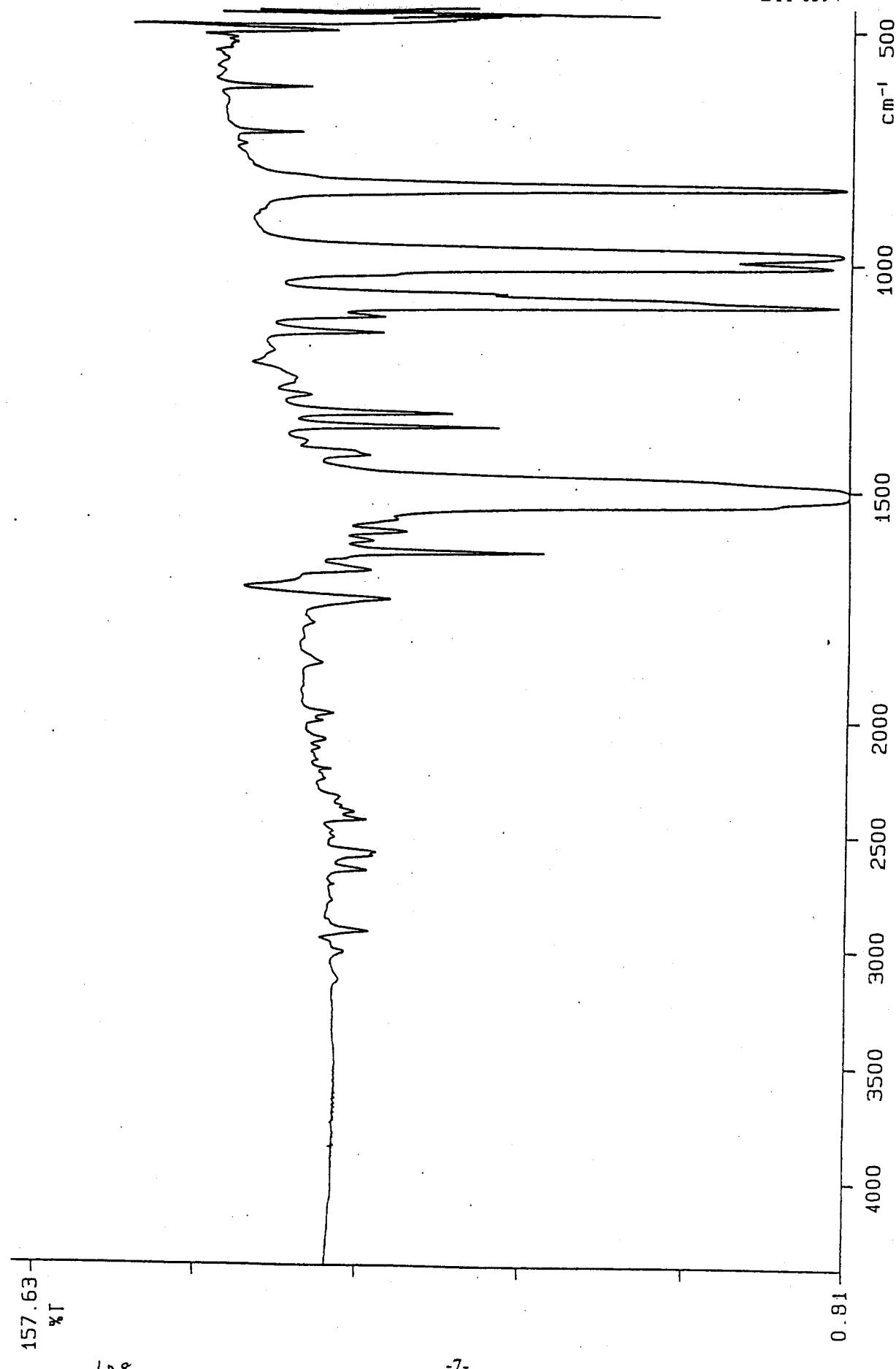


Fig. 3 IR spectrum provided by the sponsor

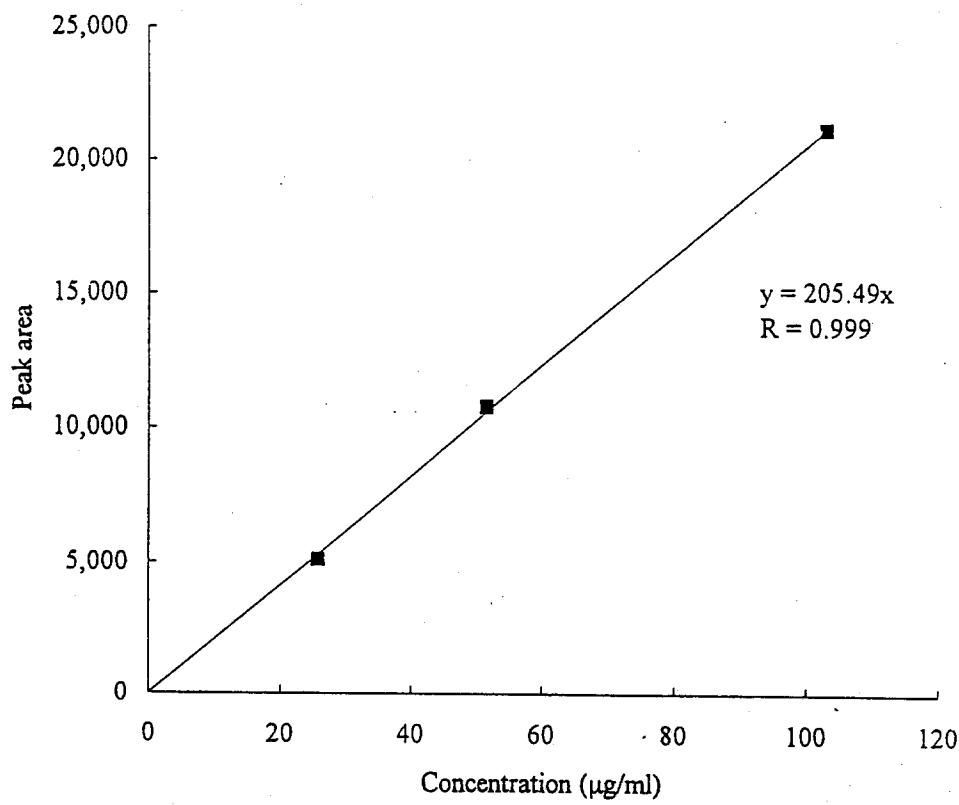


Fig.4 Calibration curve

Concentration ($\mu\text{g}/\text{ml}$)	Peak area
25.8	5,088
51.5	10,742
103	21,140

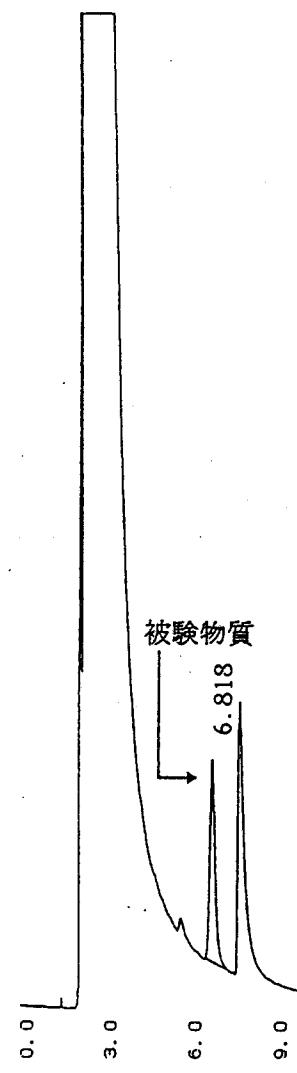


Fig.5 Typical chromatogram

Table 1 Chemical stability of the test substance in dose formulations

Time point of measurement	Nominal conc.(w/v%)	Found conc. (μ g/ml)	Dilution rate	Actual conc. (w/v%)	Mean conc. (w/v%)	R.P. (%)
Immediately after preparation	10.0	48.8855	2,000	9.78		-
		52.1958	2,000	10.4	10.1	
		50.1711	2,000	10.0		
	0.05	46.6763	10	0.0467		
		47.0703	10	0.0471	0.0480	-
		50.0835	10	0.0501		
3 days after preparation	10.0	48.4762	2,000	9.70		
		48.7190	2,000	9.74	9.78	96.8
		49.4710	2,000	9.89		
	0.05	46.0129	10	0.0460		
		46.1495	10	0.0461	0.0465	96.9
		47.3131	10	0.0473		
7 days after preparation	10.0	49.5660	2,000	9.91		
		50.7775	2,000	10.2	10.1	100
		50.4915	2,000	10.1		
	0.05	46.7729	10	0.0468		
		47.9553	10	0.0480	0.0471	98.1
		46.4766	10	0.0465		

R.P. (%) : Rate to the concentration measured immediately after preparation

Continuation Sheet of "8.0 ADDITIONAL/OPTIONAL STUDY COMMENTS"

Additinal Study and Regal Status of BPFB

1 Additional Study

STUDY	RESULTS	METHODS	LABORATORY
CORROSIVE AND IRRITANT PROPERTIES Primary eyes irritation test in rabbits	Slightly irritation	OECD 405 GLP	
CORROSIVE AND IRRITANT PROPERTIES Primary skin irritation test in rabbits	Slightly irritation	OECD 404 GLP	
ACUTE TOXICITY: RATS	LD50: 2000mg/kg≤	OECD 401 GLP	
BIODEGRADABILITY	Not biodegradable	GLP ³⁾	
BIOACCUMULATION: Cyprinus carpio	Low Accumulation Tlm ₄₈ 20mg/l(Carp)	GLP ³⁾	
Mutagenicity test Reverse-mutation assay in bacteria	Negative	GLP ³⁾	
Mutagenicity test Chromosomal aberration test in cultured mammalian cells(CHL cells)	Positive (Metabolic activation method) D ₂₀ 590 µg/ml.	GLP ³⁾	

3)Test method : According to Law Concerning the Examination and Regulation of Manufacture, etc., of Chemical Substances Japan.

2 Regal status of BPFB (Bromopentafluorobenzene) in US(TSCA), EU, and Japan

US(TSCA)	ON TSCA INVENTORY (CA RN344-04-7)
EU	EINECS No.206-449-0
JAPAN	

Receipt No. T95-2349
Report No. T-4810

STUDY CODE : K06-0565

FINAL REPORT

CHROMOSOMAL ABERRATION TEST OF BPFB USING CULTURED MAMMALIAN CELLS

May, 1997

I, the undersigned, hereby declare that this report provides a correct English translation of the final report (Study Code K06-0565, issued on May 12, 1997).

June 30, 1997

Date

QUALITY ASSURANCE STATEMENT

Sponsor:

Title: Chromosomal Aberration Test of BPFB Using Cultured Mammalian Cells
Study code: K06-0565

This report was audited by the Quality Assurance Section.
I, the undersigned, hereby declare that this report reflects
the original Japanese report.

(Date) June 30, 1997

(Signature)

Section Chief, Quality Assurance

GLP STATEMENTSponsor:Title: Chromosomal Aberration Test of BPFB Using Cultured Mammalian CellsStudy Code: K06-0565

I, the undersigned, hereby declare that this study was conducted in compliance with "Concerning Testing Facilities Stipulated in Article 4 of the Order Prescribing the Items of the Test Related to the New Chemical Substances and of the Toxicity Investigations Related to the Designated Chemical Substances (Notification No. 39 of the Planning and Coordination Bureau, Environment Agency (EA), No. 229 of the Pharmaceutical Affairs Bureau, Ministry of Health and Welfare (MHW) & No. 85 (1984) of the Basic Industries Bureau, Ministry of International Trade and Industry (MITI), March 31, 1984; Notification No. 233 of the Planning and Coordination Bureau, EA, No. 38 of the Pharmaceutical Affairs Bureau, MHW & No. 823 (1988) of the Basic Industries Bureau, MITI, revised on November 18, 1988)" and with "Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice (May 12, 1981)".

Management: Signed in original May 12, 1997

QUALITY ASSURANCE STATEMENTSponsor:Title: Chromosomal Aberration Test of BPFB Using Cultured Mammalian CellsStudy Code: K06-0565

This study was audited by Quality Assurance Section and the study procedures were inspected on the following dates.

Dates of Inspections and Audits	Dates of Reports to Study Director	Dates of Reports to Management
November 28, 1996	November 28, 1996	November 29, 1996
March 11, 1997	March 11, 1997	March 11, 1997
March 31, 1997	April 7, 1997	April 7, 1997
May 9, 1997	May 9, 1997	May 9, 1997
May 12, 1997	May 12, 1997	May 12, 1997

I, the undersigned, hereby declare that this report provides an accurate description of the methods and procedures used in this study and that the reported results accurately reflect the raw data obtained.

Section Chief, Quality Assurance: Signed in original May 12, 1997

Study Code: K06-0565
Test Substance Code: HR3322
Sponsor Code: N-080

TITLE

Chromosomal Aberration Test of BPFB Using Cultured Mammalian Cells

SPONSOR

TESTING FACILITY

PURPOSE OF STUDY

The purpose of this test is to determine the mutagenic potential of the test substance using a cell line of Chinese hamster lung fibroblasts (CHL cells).

TESTING METHOD

This study was conducted in accordance with 'Notification on Partial Revision of Testing Methods Relating to the New Chemical Substances (Notification No. 700 of the Planning and Coordination Bureau, EA, No. 1039 of the Pharmaceutical Affairs Bureau, MHW & No. 1014 (1986) of the Basic Industries Bureau, MITI, December 5, 1986)'.

GLP COMPLIANCE

This study was conducted in compliance with "Concerning Testing Facilities Stipulated in Article 4 of the Order Prescribing the Items of the Test Related to the New Chemical Substances and of the Toxicity Investigations Related to the Designated Chemical Substances (Notification No. 39 of the Planning and Coordination Bureau, EA, No. 229 of the Pharmaceutical Affairs Bureau, MHW & No. 85 (1984) of the Basic Industries Bureau, MITI, March 31, 1984; Notification No. 233 of the Planning and Coordination Bureau, EA, No. 38 of the Pharmaceutical Affairs Bureau, MHW & No. 823 (1988) of the Basic Industries Bureau, MITI, revised on November 18, 1988)" and with "OECD Principles of Good Laboratory Practice (May 12, 1981)".

PERIOD OF STUDY

Commencement of Study: November 28, 1996
Initiation of Preliminary Test: December 16, 1996
Completion of Observation: April 28, 1997
Completion of Study: May 12, 1997

LOCATION AND PERIOD FOR RETENTION OF RAW DATA AND SPECIMENS

Data and specimens, and each remaining lot of the test substance will be retained in the archives and test substance storage room, respectively, of Hita Research Laboratories for 10 years following the date of completion of the study. After termination of the retention period, any measures taken will be done so with the approval of the sponsor. Samples and specimens that are liable to deteriorate markedly will be retained only for as long as the quality of the preparation permits evaluation with the sponsor's consent.

AUTHOR AND PERSONS CONCERNED WITH STUDY

Study Director: Signed in original May 12, 1997

Study Staff:

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SUMMARY

The effect of BPFB on the chromosomal aberration was investigated using Chinese hamster lung fibroblasts (CHL cells) for 24 h and 48 h treatments (direct method) and 6 h treatment with and without S9 Mix (metabolic activation method).

Cell growth inhibition test and cell division inhibition test were carried out to determine the dose levels of the test substance. From the results of these tests, chromosomal aberration tests were carried out using 95, 190 and 380 $\mu\text{g}/\text{ml}$ of the test substance for 24 and 48 h treatments by the direct method, 137.5, 275 and 550 $\mu\text{g}/\text{ml}$ in the group without S9 Mix and 400, 500 and 600 $\mu\text{g}/\text{ml}$ in the group with S9 Mix by the metabolic activation method.

As the results, the incidence of structural chromosomal aberration in the group with S9 Mix by the metabolic activation method increased at 400-500 $\mu\text{g}/\text{ml}$ of the test substance, but it decreased to 4.5% at 600 $\mu\text{g}/\text{ml}$. Because of this result without dose-relationship, a reexamination was conducted with the same dose as the chromosomal aberration test. The result showed that the test substance induced structural chromosomal aberrations dose-dependently within the dose range of 400-600 $\mu\text{g}/\text{ml}$ in the group with S9 Mix by the metabolic activation method. On the other hand, MMC and CPA induced evident chromosomal aberrations.

It is concluded that BPFB induced chromosomal aberration under the present test conditions.

D_{20} value of the test substance was calculated as 590 $\mu\text{g}/\text{ml}$.

MATERIALS AND METHODS

1. TEST SUBSTANCE AND POSITIVE CONTROLS

1.1 Test Substance (Information Provided by the Sponsor)

1) Name

Bromopentafluorobenzene

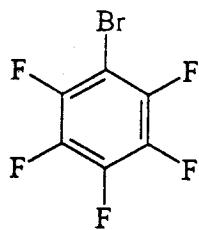
Other Name: BPFB

CAS No.: 344-04-7

2) Lot No.

3) Supplier

4) Structural Formula or Rational Formula (or Outline of Manufacturing Method, in Case Both are Unknown)

(Molecular Formula: C₆BrF₅)

5) Purity

99.9 w/w%

6) Name and Concentration of Impurities

Pentafluorobenzene 0.1 w/w%

7) Physicochemical Properties

Appearance at Ordinary Temperature: Liquid

Molecular Weight: 246.96

Stability Stable

Melting Point: -31°C

Boiling Point: 137°C

Vapor Pressure: —

Partition Coefficient: —

Density: 1.981

Solubility: Oil soluble

Degree of Solubility: Water: —

DMSO: —

Acetone: —

Others: —

8) Storage Conditions

Stored in a cold and dark place

9) Care on Handling

Gloves, a mask, a head cap and a lab coat were worn.

1.2 Positive Control Substances

1) Mitomycin C (MMC)

Manufacturer: Kyowa Hakko Kogyo Co., Ltd.

Lot No.: 136AFK

Appearance: royal purple crystals or crystalline powder

Composition: 2 mg titer of J.P. MMC and 48 mg of J.P. sodium chloride in one vial

2) Cyclophosphamide monohydrate (CPA)

Manufacturer: Wako Pure Chemical Industries, Ltd.

Lot No.: PTL7433

Appearance: white crystalline powder

Purity: 98.8%

3) Storage Conditions

MMC and CPA were stored at room temperature and in a refrigerator, respectively.

4) Care on Handling

Gloves, a mask, a head cap and a lab coat were worn.

2. CELLS AND MEDIUM

2.1 Cell Line and Reason for Selection

Chinese hamster lung fibroblasts (CHL cells, clone No. 11) were used in this test because they are widely employed for *in vitro* chromosomal aberration tests, and they show quite high sensitivity to chemical mutagens. A large amount of data is also available about their chromosomal aberrations. CHL cells (passage number 18) were supplied by the National Institute of Hygienic Sciences on September 28, 1988. The modal number of chromosomes is 25 per cell. The time required for doubling of cell number is about 15 h. CHL cells preserved by freezing were defrosted and cultured. The passage number of cells used in the chromosomal aberration test for all groups was 24, and that in the group with S9 Mix by the metabolic activation method in the reexamination was 28.

2.2 Medium and Pure Water

Eagle's minimum essential medium (Lot Nos. 366603, 372607 and 373609, Nissui Seiyaku Co., Ltd.) was supplemented with newborn calf serum (Lot No. NBM05, Mitsubishi Kasei Corporation) at a rate of 10 v/v%. This medium is referred to hereafter as 10% NCS/MEM.

Distilled water for preparation of the medium was obtained with a glass still (WG-32 and WG220, Yamato Scientific Co., Ltd.).

3. S9 MIX

3.1 Rat Liver S9 (Kikkoman Co., Ltd.)

Induction method: Sprague-Dawley rats, 7-week-old, male (198-243 g (RAA-351), 190-226 g (RAA-356)), were intraperitoneally administrated phenobarbital (30 mg/kg×1 time, 60 mg/kg×3 times) and 5,6-benzoflavone (80 mg/kg×1 time).

Lot No.: RAA-351 (manufacturing date: September 6, 1996; purchase date: October 2, 1996)

RAA-356 (manufacturing date: December 13, 1996; purchase date: February 4, 1997)

Storage: -80°C (Deep freezer, ULT-1285, REVCO Co., Ltd.)

3.2 Composition of S9 Mix

One ml of S9 Mix composes of 4 µmol HEPES (pH 7.2), 5 µmol MgCl₂, 33 µmol KCl, 5 µmol G-6-P, 4 µmol NADP and 0.3 ml S9.

3.3 Components of S9 Mix/MEM

S9 Mix 0.5 ml

10% NCS/MEM 2.5 ml

4. CELL CULTURE

Five ml of 10% NCS/MEM containing 1.5×10^4 or 0.5×10^4 cells/ml was plated in a 60 mm diameter Petri dish (Iwaki Glass Co., Ltd.) and a culture flask with the base area of 25 cm² (Greiner Labortechnik Co., Ltd.) and cultured for two or three days. Monolayer cells in logarithmic growth phase were exposed to the test substance two or three days after culturing.

Cell culture was carried out in a CO₂ incubator under the following conditions.

Temperature: 37±0.5°C

Humidity: almost 100%

Atmosphere: air containing 5% CO₂

5. PREPARATION OF THE TEST SUBSTANCE AND THE POSITIVE CONTROLS

5.1 Test Substance

1) Preparation

Because the test substance dissolved more than 500 mg/ml in dimethylsulfoxide (DMSO, Lot Nos. DM100 and DP100, Dojin Laboratories), DMSO was used as a solvent. The original solution was diluted with the same solvent to make the required concentrations.

2) Preparation Time

Test substance solutions were prepared immediately before use and used within 2 h.

5.2 Added Amount of Test Substance Solutions

To the medium, 1% solution was added.

5.3 Positive Controls

1) Preparation

Positive control substances were dissolved in distilled water, and diluted with the same solvent to make the required concentrations.

2) Preparation Time

Positive control substance solutions were prepared immediately before use and used within 2 h.

6. PREPARATION OF MICROSCOPE SLIDES

Cells were removed from each dish with 2 ml of 0.25% trypsin, and collected by centrifugation in a tube. Then, 0.075 M KCl was added to the tube, and hypotonic treatment was carried out at 37°C for 15 min. Methanol-acetic acid (3:1) solution was then poured into the tube to fix the cells and make a slightly cloudy cell suspension, which is then plated onto microscope slides and spread. The cells on each slide were finally dried and stained with 2% Giemsa solution. Two slides per dish were prepared.

7. DOSE DETERMINATION TEST

In the cell growth inhibition test, the 50% growth inhibition concentrations of the test substance were determined. In the cell division inhibition test, the highest concentrations with sufficient metaphase cells which are practically scorable were determined as the maximum dose for the chromosomal aberration test.

7.1 Cell Growth Inhibition Test

In the direct method, 5 ml of 10% NCS/MEM including the test substance was added to each dish after removal of the medium from two or three-day-old culture, followed by culture for 24 or 48 h.

In the metabolic activation method, 3 ml of 10% NCS/MEM (the group without S9 Mix) and S9 Mix/MEM (the group with S9 Mix) including the test substance were added to each dish after removal of the medium, and treated for 6 h. After S9 Mix/MEM including the test substance was removed, dishes were rinsed three times with 2 ml of Ca^{2+} , Mg^{2+} -free phosphate-buffered saline [PBS(-)]. Then, 5 ml of 10% NCS/MEM was added to each dish, and the cells were cultured for 18 h.

In both methods, two hours before the end of incubation, colcemid was added to the medium to give a final concentration of 0.1 $\mu\text{g}/\text{ml}$.

After removal of the medium at the end of incubation, the cells were detached from each dish with 2 ml of 0.25% trypsin, and counted using a Microcell Counter F-500 (Toa Medical Electronics Co., Ltd.). Two dishes were used for each dose, and the cells were counted in each respective dish.

7.2 Cell Division Inhibition Test

Observations of the amount of mitotic metaphase and chromosomal aberration were conducted using preparation slides of cells treated in the cell growth inhibition test.

8. CHROMOSOMAL ABERRATION TEST

8.1 Dose Levels

Test Substance: The highest concentration decided in the dose determination test was employed as the maximum dose for the chromosomal aberration test. The test was carried out at three dose levels prepared by diluting the maximum dose using a geometric progression or a same dose difference.

Positive Controls: The following concentrations were used in the positive controls based on our background data.

Direct Method:

24 h treatment:	MMC	0.05	$\mu\text{g}/\text{ml}$
48 h treatment:	MMC	0.05	$\mu\text{g}/\text{ml}$

Metabolic Activation Method:

without S9 Mix:	MMC	0.1	$\mu\text{g}/\text{ml}$
with S9 Mix:	CPA	10	$\mu\text{g}/\text{ml}$

8.2 Method

Cell cultures and treatments were done using the same procedures as those for the cell growth inhibition test and chromosome slides were prepared.

A solvent-treated group and a MMC and CPA-treated group were used as a negative and a positive control, respectively.

8.3 Number and Identification of Dishes

Two dishes were chosen at random to minimize any variation in results and used for each dose. Preparation was conducted in each respective dish. The test code number, treatment method and dose level were noted on the dishes for identification of each culture.

9. OBSERVATION AND SCORING OF CHROMOSOMAL ABERRATIONS

The numbers of cells with chromatid or chromosome-type structural aberrations (such as gaps, breaks, exchanges, etc.) and with numerical aberrations (polyploid, endo-reduplication) were checked by observing 100 metaphases for each dish. The incidences of cells with numerical and/or structural aberrations were obtained by observing 200 metaphases for each group. The incidence of structural aberration was calculated with and without gaps, respectively.

A gap was scored when a clear discontinuity (larger than a chromatid width) was evident, and when the distal part of the chromatid or chromosome showed no dislocation. Slides were all coded and blind-tested by microscopy.

10. JUDGEMENT OF RESULTS

The results obtained were assessed as follows. The incidence of cells (mean value for two dishes) with aberrations including gaps was

less than 5%	negative (-)
5% or more and less than 10%	suspect positive (\pm)
10% or more and less than 20%	positive (+)
20% or more and less than 50%	positive (++)
50% or more	positive (+++)

The test substance is judged to be positive for induction of chromosomal aberration when the incidence of 10% or more was dose-related or reproducible. If it was judged to be positive, the D_{20} value, which showed a dose being inducible the chromosomal aberration of 20%, was calculated. Reexamination was performed whenever the result was suspect positive, positive at only one dose level, or not evidently dose-related.

UNEXPECTED SITUATIONS AND DEVIATIONS FROM PROTOCOL

There were no unexpected situations which might have affected the test results and deviation from protocol.

RESULTS

The values for two dishes were not markedly different, the incidence of cells with any aberration did not exceed 5% in the negative control, the incidence of cells with structural aberrations excluding gap was 10% or more in the positive control. There were no fluctuations in the test conditions and no contamination with microorganisms in the cultures which might have affected the results. The test results were therefore considered to be valid.

1. CELL GROWTH INHIBITION TEST AND CELL DIVISION INHIBITION TEST (Table 1 and Fig. 1)

Because initial cell growth inhibition tests using Petri dish did not show an accurate dose-cytotoxicity relationship of the test substance, culture flasks were used in place of Petri dishes with consideration for boiling point of the test substance. Dose levels used in the cell growth inhibition test and the cell division inhibition test were shown in Table 1. The growth rate in the solvent control was considered to be 100%. Fifty percent growth inhibition concentrations of the test substance were about 370 µg/ml in 24 h and 48 h treatments by the direct method, and about 510 µg/ml in the group without S9 Mix and about 590 µg/ml in the group with S9 Mix by the metabolic activation method. Mitotic metaphases of chromosomes sufficient for assessing chromosomal aberration were observed at doses of 380 µg/ml or less in 24 h and 48 h treatments by the direct method, and 550 µg/ml or less in the group without S9 Mix and 600 µg/ml or less in the group with S9 Mix by the metabolic activation method. In the group with S9 Mix by the metabolic activation method, an increase of the structural aberration was observed at 400-600 µg/ml of the test substance in the specimen of the cell division inhibition test.

Based upon the results, the following 3 dose levels were employed in each method of chromosomal aberration test.

24 h treatment by the direct method:	95, 190 and 380 µg/ml
48 h treatment by the direct method:	95, 190 and 380 µg/ml
Without S9 Mix by the Metabolic activation method:	137.5, 275 and 550 µg/ml
With S9 Mix by the Metabolic activation method:	400, 500 and 600 µg/ml

2. CHROMOSOMAL ABERRATION TEST

2.1 Direct Method (Table 2 and Fig. 2)

1) 24 h Treatment

The incidences of cells with structural aberrations including gap were 1.0% at 95 µg/ml, 1.5% at 190 µg/ml and 4.0% at 380 µg/ml. The incidence in the solvent control was 0.5%, i.e. within the normal range. The positive control treated with MMC showed the structural aberration of 52.5%.

The incidences of polyploid cells at any doses were less than 5%.

2) 48 h Treatment

The incidences of cells with structural aberrations including gap were 1.5% at 95 µg/ml, 1.5% at 190 µg/ml and 2.0% at 380 µg/ml. The incidence in the solvent control was 1.5%, i.e. within the normal range. The positive control treated with MMC showed the structural aberration of 46.0%.

The incidences of polyploid cells at any doses were less than 5%.

2.2 Metabolic Activation Method (Table 3 and Fig. 3)

1) Without S9 Mix

The incidences of cells with structural aberrations including gap were 1.5% at 137.5 µg/ml, 1.0% at 275 µg/ml and 0.5% at 550 µg/ml. The incidence in the solvent control was 0.5%, i.e. within the normal range. The positive control treated with MMC showed the structural aberration of 33.0%.

The incidences of polyploid cells at any doses were less than 5%.

2) With S9 Mix

The incidences of cells with structural aberrations including gap were 5.0% at 400 µg/ml, 23.0% at 500 µg/ml and 4.5% at 600 µg/ml. The incidence in the solvent control was 1.0%, i.e. within the normal range. The positive control treated with CPA showed the structural aberration of 36.5%.

The incidences of polyploid cells at any doses were less than 5%.

2.3 Reexamination with S9 Mix by the Metabolic Activation Method (Table 4 and Fig. 4)

Though the incidence of structural chromosomal aberration in the group with S9 Mix by the metabolic activation method increased at 400-500 µg/ml of the test substance in the chromosomal aberration test, it decreased to 4.5% at 600 µg/ml. Because of this result without dose-relationship, a reexamination was conducted with the same dose as the chromosomal aberration test.

1) With S9 Mix

The incidences of cells with structural aberrations including gap were 12.5% at 400 µg/ml, 15.5% at 500 µg/ml, and 19.0% at 600 µg/ml.

The incidence in the solvent control was 0.5%, i.e. within the normal range. The positive control treated with CPA showed the structural aberration of 25.0%.

The incidences of polyploid cells at any doses were less than 5%.

CONCLUSION

Chromosomal aberration test of BPFB was carried out using CHL cells.

Though the incidence of structural chromosomal aberration in the group with S9 Mix by the metabolic activation method increased at 400-500 µg/ml of the test substance in the chromosomal aberration test, it decreased to 4.5% at 600 µg/ml. Because of this result without dose-relationship, a reexamination was conducted with the same dose as the chromosomal aberration test.

As the result of the test, the test substance induced structural aberrations dose-dependently in the dose range of 400-600 µg/ml.

It is concluded that BPFB induced chromosomal aberration under the present test conditions.

D₂₀ values of the chromosomal aberration test and the reexamination with S9 Mix by the metabolic activation method were 1,000 µg/ml (S=140) and 590 µg/ml (S=39), respectively. Therefore, 590 µg/ml was adopted as a final D₂₀ value.

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Table 1 Results of the dose determination test of BPFB

		Concentration of the test substance($\mu\text{g/ml}$)	0	200	*	250	*	300	*	320	*	340	*	360	*	380	*	400	*	500
		Growth rate *1 (%)	100	80.7	79.3	77.3	71.4	60.3	51.8	47.3	22.7									
		Index amount of mitotic metaphase *2	++	++	++	++	++	++	++	++	+	±	-							
Direct method	24 h treatment	Chromosomal aberrations *3 (%)	polyploid	0	0	0	2	2	2	0	0	0	0	0	0	0	0	0		
	48 h treatment	Chromosomal aberrations *3 (%)	structural	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0		
without S9 Mix	24 h treatment	Concentration of the test substance($\mu\text{g/ml}$)	0	200	250	300	320	340	360	380	400	400	400	400	400	400	400	400	500	
	48 h treatment	Concentration of the test substance($\mu\text{g/ml}$)	0	200	250	300	320	340	360	380	380	380	380	380	380	380	380	380	500	
Metabolic activation method	24 h treatment	Growth rate *1 (%)	100	61.6	64.8	62.9	56.6	61.9	58.8	29.6	24.8	24.8	24.8	24.8	24.8	24.8	24.8	24.8	7.9	
	48 h treatment	Growth rate *1 (%)	100	92.8	89.4	88.9	86.0	57.3	47.2	8.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.1	
with S9 Mix	24 h treatment	Index amount of mitotic metaphase *2	++	++	++	++	++	++	++	++	+	+	+	+	+	+	+	+	-	
	48 h treatment	Index amount of mitotic metaphase *2	++	++	++	++	++	++	++	++	+	+	+	+	+	+	+	+	-	

*1 Mean value for two dishes.

*2 Judgement

++ : abundant, + : a few, ± : rare, - : none

*3 Number of observation were 50 cells.

*Precipitation of the test substance was observed in culture medium.

Table 2 Results of chromosomal aberration test (without metabolic activation)

Name of test substance : BPFB	Treatment time(h)	Concen- tration ($\mu\text{g}/\text{ml}$)	Observed	Number and the percentages(%) of cells showing structural chromosomal aberrations							Judge- ment	
				Number of polyploids	Judge- ment	Gap	ctb	Chromatid-type	cse	Others	Total	
Solvent (DMSO)	24	0	100	0		0	1		0	0	1	1
		200	0(0.0)			0	0	0(0.0)	0(0.0)	0	0	0
	48	0	100	1		1	0	0(0.0)	0(0.0)	0	1(0.5)	1(0.5)
		200	1(0.5)			0	1	1(0.5)	0(0.0)	0	0	1
	24	95 *	100	1	-	0	0	0(0.0)	1(0.5)	0(0.0)	1(1.0)	1
		200	2(1.0)			0(0.0)	1	0(0.0)	0(0.0)	1(0.5)	0(0.0)	2(1.0)
	48	190 *	100	0	-	0	0	0(0.0)	0(0.0)	0	0	0
		200	1(0.5)			0	3	0(0.0)	1(0.5)	0(0.0)	3	3
	24	380 *	100	2		0	1	0(0.0)	3(1.5)	0(0.0)	3(1.5)	-
		200	2(1.0)			0	1	2	0	2	2	5
	48	95 *	100	0	-	0	2	2(1.0)	2(1.0)	2(1.0)	0(0.0)	8(4.0)
		200	0(0.0)			0(0.0)	2	0(0.0)	0(0.0)	0	0	3
Test Substance	24	190 *	100	3	-	0	1	0(0.0)	1(0.5)	0(0.0)	3(1.5)	3
		200	3(1.5)			0	0	1(0.5)	0(0.0)	0	1(0.5)	2
	48	380 *	100	0	-	0	1	1(0.5)	0(0.0)	0	1(0.5)	1
		200	1(0.5)			1	2	0	0	0	0	-
	24	0.05	100	0	-	0	2	0	1	0	3	3
		200	0(0.0)			0(0.0)	2(1.0)	0(0.0)	1(0.5)	0(0.0)	3(1.5)	3
Positive control (MMC)	48	0.05	100	1	-	0	5	0(0.0)	34	0	36	36
		200	0(0.5)			1	29	44	0	0	56	56
	24	0.05	100	0	-	1	34(17.0)	78(39.0)	0(0.0)	0(0.0)	92(46.0)	92(46.0)
		200	1(0.5)			1	34(17.0)	78(39.0)	0(0.0)	0(0.0)	92(46.0)	92(46.0)

*: total number and percentage of cells except those which has only gaps +g: total number and percentage of cells including those which has only gaps
 ctb: chromatid break cte: chromatid exchange est: chromosome break cse: chromosome exchange other: fragmentation etc.(except pulverization)
 MMC: Mitomycin C

* Precipitation of the test substance was observed in culture medium.

Table 3 Results of chromosomal aberration test (with metabolic activation)

Treatment	S9 Mix	Concen- tration ($\mu\text{g/ml}$)	Number of cells Observed	Number of polyploids	Number and the percentages(%) of cells showing structural chromosomal aberrations						Judge- ment	
					gap	ctb	cte	csb	cse	Others	Total	
Solvent (DMSO)	-	0	100	1	0	0	0	0	0	0	0	-
	+	0	100	0	0	0	0	0	1	0	1	-
	-	137.5*	100	1	0	0	0	0	1(0.5)	1(0.5)	1(0.5)	-
	-	275*	100	1	0	0	0	0	0	0	0	-
	-	550*	100	2	0	0	0	0	0	0	0	-
	-	400*	100	2	0	0	0	0	0	0	0	-
	+	500*	100	2	1	19	17	0	0	0	28	++
	+	600*	100	0	0	8	16	0	0	0	17	++
	-	Positive control (MMC)	100	1	1	2	3	0	0	0	45(22.5)	46(23.0)
	+	Positive control (CPA)	100	1	0	2	2	0	0	0	5	-

Amount of S9(5%), Treatment time of the test substance(6h), Recovery time of the test substance(18h)

-g: total number and percentage of cells except those which has only gaps +g: total number and percentage of cells including those which has only gaps
 ctb: chromatid break cte: chromatid exchange csb: chromosome break cse: chromosome exchange other: fragmentation etc.(except pulverization)

MMC: Mitomycin C

CPA: Cyclophosphamide, monohydrate

* Precipitation of the test substance was observed in culture medium.

Table 4 Results of reexamination (with metabolic activation)

Name of test substance: BPFB	S9 Mix	Concen- ration ($\mu\text{g/ml}$)	Observed Number of polyploids	Number of cells judged ment	Gap	Chromatid-type	Chromosome-type	Others	Total	Judge- ment
Treatment					g	ctb	cte	cse	-g	+g
Solvent (DMSO)	+	0	100 100 200	1 0 1(0.5)	0 0 0(0.0)	1 0 1(0.5)	0 0 0(0.0)	0 0 0(0.0)	1 0 1(0.5)	1 0 1(0.5)
Test Substance		400 *	100 200	2 3(1.5)	- 5(2.5)	5 6(3.0)	13 14(7.0)	1 1(0.5)	0 4(2.0)	0 0(0.0)
	+	500 *	100 200	2 2(1.0)	- 2(1.0)	1 5(2.5)	10 21(10.5)	0 1(0.5)	4 5(2.5)	7 0(0.0)
		600 *	100 200	1 2(1.0)	- 0	0 13	9 11	0 0	0 0	22(11.0) 25(12.5)
Positive control (CPA)	+	10	100 200	0 1(0.5)	- 3(1.5)	2 5	18 18	0 1	0 1	24 0(0.0)
						11(5.5)	36(18.0)	0(0.0)	0 1(0.5)	47(23.5) 50(25.0)

Amount of S9(5%), Treatment time of the test substance(6h), Recovery time of cells after treatment of the test substance(18h)

-g: total number and percentage of cells except those which has only gaps +g: total number and percentage of cells including those which has only gaps
 ctb: chromatid break cte: chromatid exchange csb: chromosome break cse: chromosome exchange other: fragmentation etc.(except pulverization)

CPA: Cyclophosphamide, monohydrate

* Precipitation of the test substance was observed in culture medium.

K06-0565

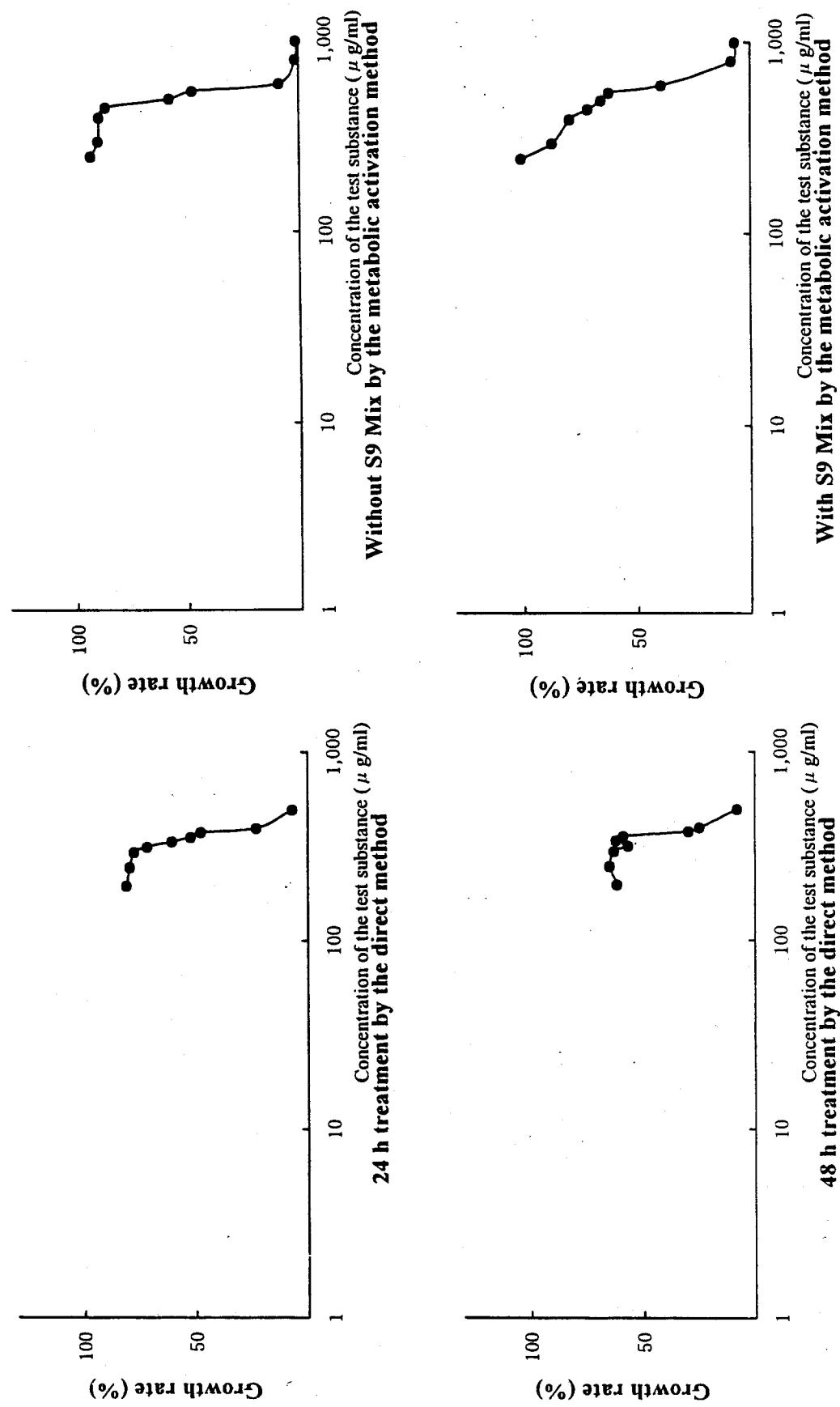


Fig. 1 Results of the dose determination test of BPFB

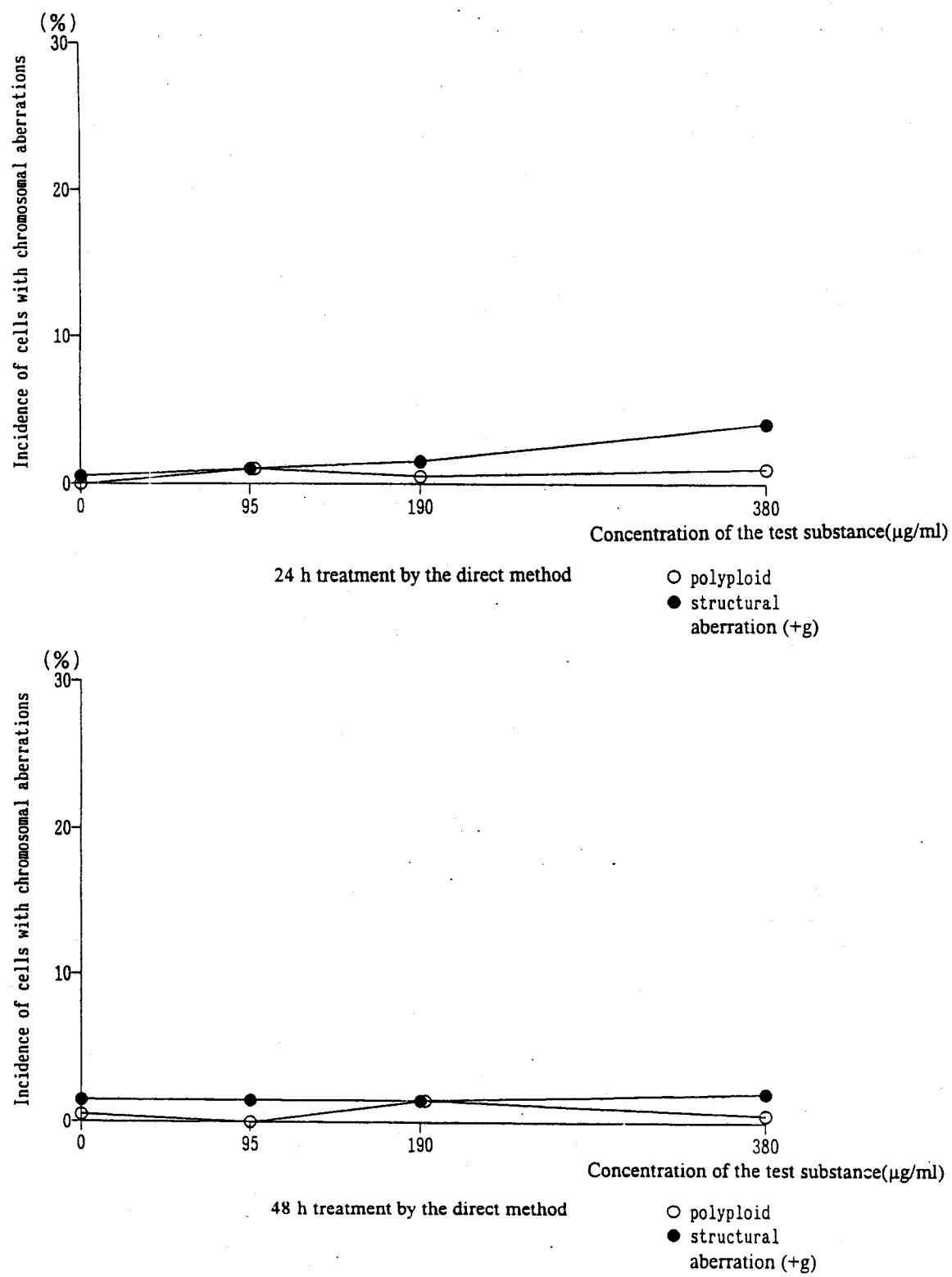


Fig.2 Results of chromosomal aberration test of BPFB

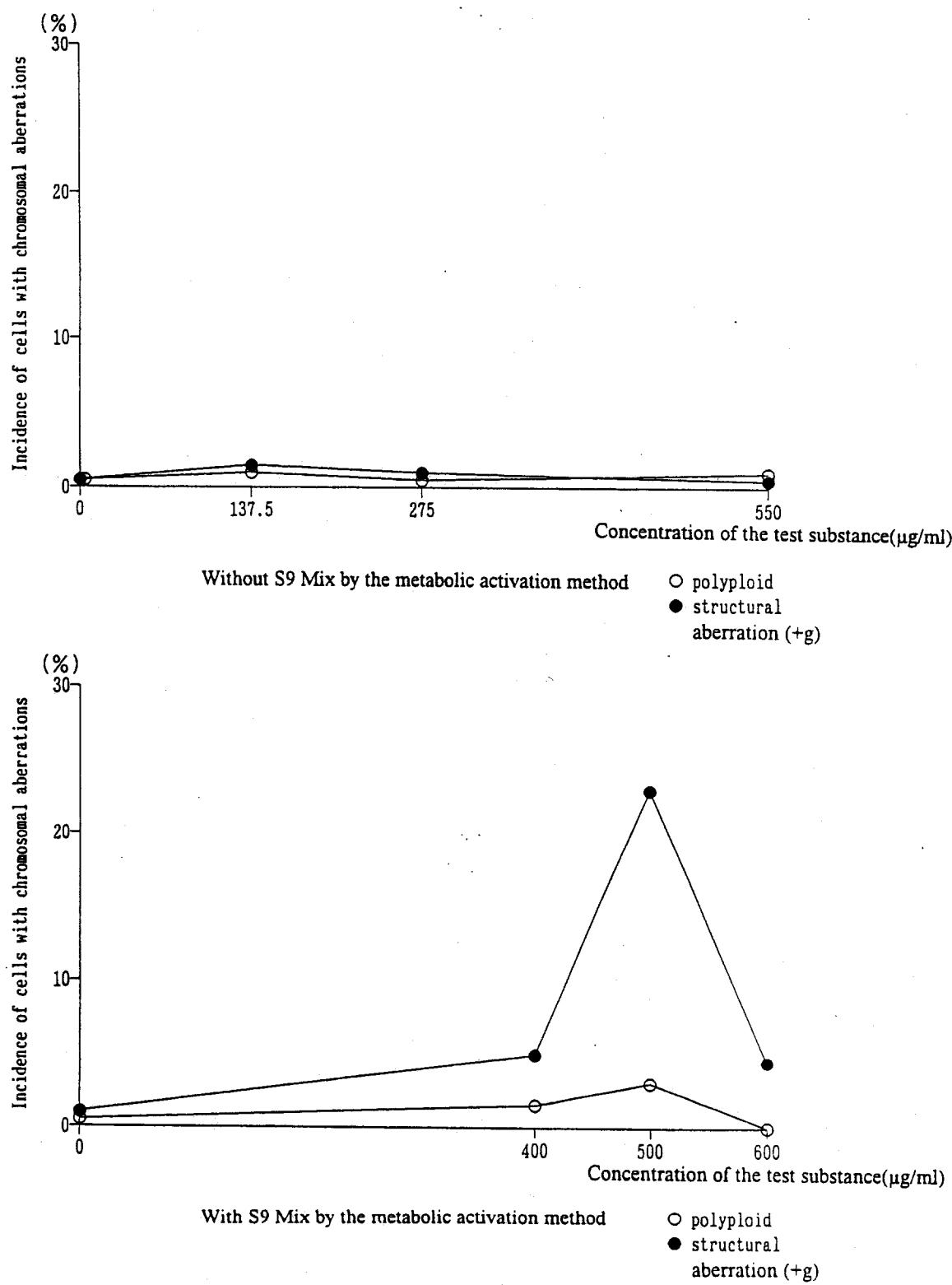


Fig.3 Results of chromosomal aberration test of BPFB

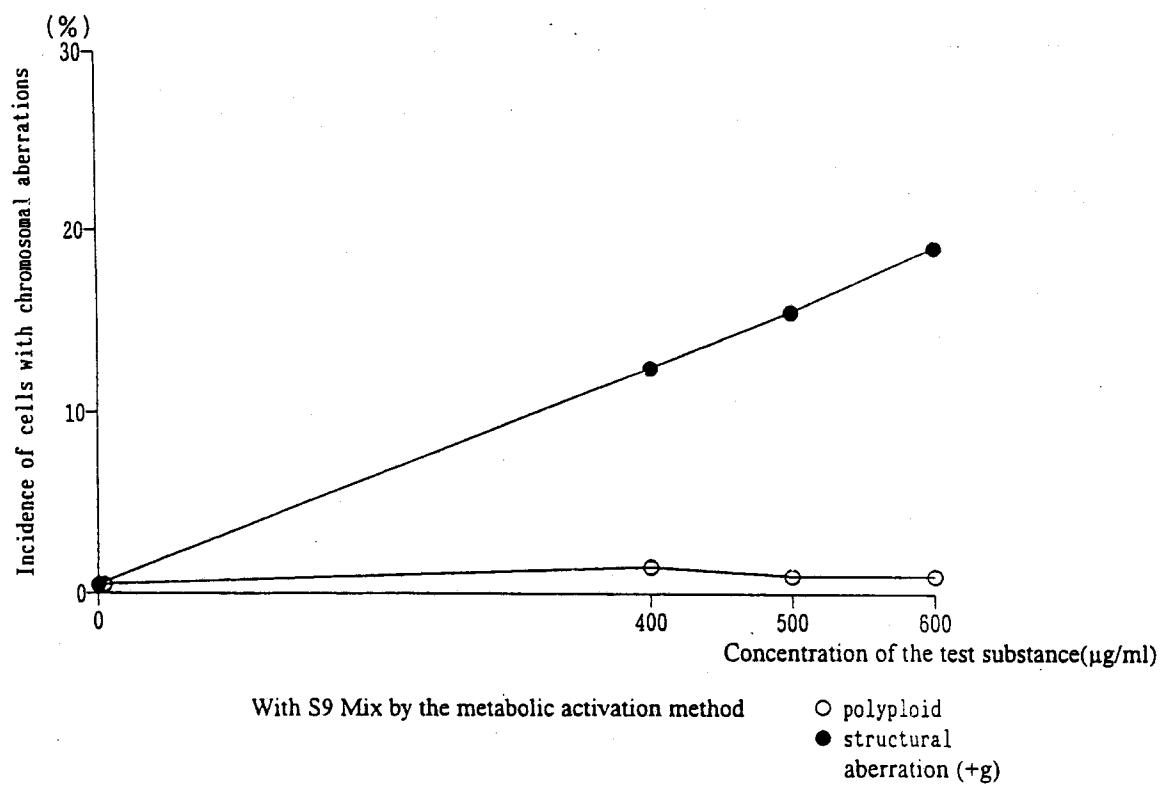
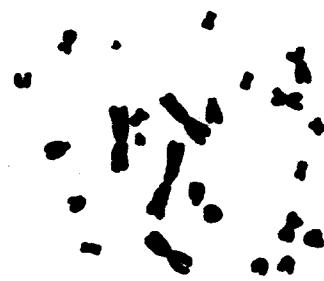
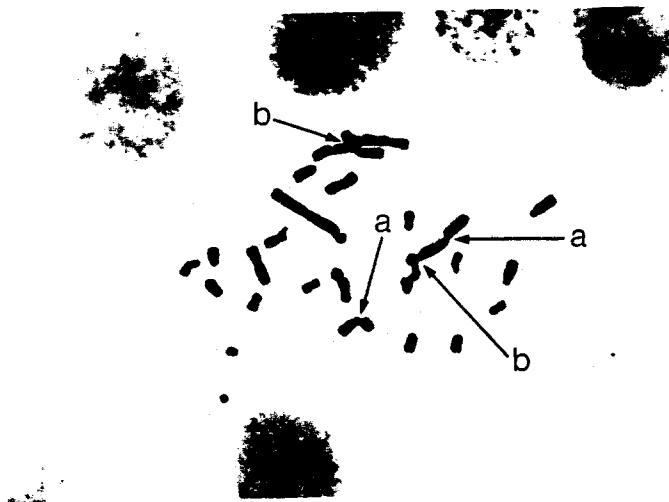


Fig.4 Results of reexamination of BPFB



対 照(代謝活性化法 S9 Mix 添加群 再試験 溶媒対照)



構造異常(代謝活性化法 S9 Mix 添加群 再試験 600 µg/ml)

a : 染色分体型切断

b : 染色分体型交換

図 5 BPFB により誘発された染色体異常

MATERIAL SAFETY DATA SHEET

DATE PREPARED:14.Feb.1996

DATE REVISED :14.Aug.1997

1.CHEMICAL PRODUCT & COMPANY IDENTIFICATION

- CHEMICAL PRODUCT NAME : BPFB
- NAME OF MANUFACTURER :
- ADDRESS :
- TEL No. :
- FAX No. :
- EMERGENCY TEL No. :

2.COMPOSITION/INFORMATION ON INGREDIENTS

- SUBSTANCE/MIXTURE : Substance
- CHEMICAL NAME : Bromopentafluorobenzene
- SYNONYMS : -
- CAS REGISTRY NUMBER : 344-04-7
- INGREDIENTS AND COMPOSITION : More Than 99%
- CHEMICAL FORMULA : C₆BrF₅
- UN CLASS UN No. : Not applicable for the definition of the dangerous goods of United Nations.

3.HAZARD IDENTIFICATION

- CLASS NAME OF HAZARDOUS CHEMICALS FOR MSDS IN JAPAN: Not Applicable
- PHYSICAL AND CHEMICAL HAZARDS:
 - Combustible liquid.
 - Contact with strong acids or bases may form toxic stench.
 - During a fire, this substance decompose and may liberate toxic fumes. (Halogen gases, COx.)
 - Contact with bases may form fluorine salts. Contact of the salts with strong acids may liberate irritating, highly toxic and corrosive HF.
- ADVERSE HUMAN HEALTH EFFECTS:
 - Slightly irritating to the skin and the eyes. This substance is believed to present very little hazard if swallowed. May cause irritating to respiratory tract.
 - Possible risk of irreversible effects to teeth by repeated oral dose.
- ENVIRONMENTAL EFFECTS:
 - This substance is not biodegradable. This substance is low bioaccumulation.

4.FIRST-AID MEASURES

- EYE CONTACT :
 - First rinse with plenty of water for several minutes(remove contact lenses if easily possible), then take to a doctor.
- SKIN CONTACT :
 - Immediately flush skin with plenty of water. Remove clothing. Get medical attention immediately.

·INHALATION :

Remove to fresh air. If not breathing, give artificial respiration. If breathing difficult, give oxygen. Get immediate medical attention.

·INGESTION :

If swallows, do not induce vomiting. Give victim a glass of water. Call physician immediately.
Never give anything by mouth to an unconscious person.

5.FIRE-FIGHTING MEASURES**·EXTINGUISHING MEDIA:**

Use alcohol foam, carbon dioxide, powder, or water spray.

·SPECIFIC HAZARDS WITH REGARD TO FIRE-FIGHTING MEASURES:

Evacuate area and fight fire from safe distance.
Firefighters should wear self-contained breathing apparatus with full face piece operated in positive pressure mode.
During a fire, this substance decomposes and may liberate toxic fumes. (Halogen gases, COx.)

6.ACIDENTAL RELEASE MEASURES

Evacuate non essential personnel. Shut off all sources of ignition. No flares, smoking or flames in area. Collect leaking and spilled liquid in sealable containers as far as possible. Do not let this chemical enter the environment. Wear suitable protective equipment.

7.HANDLING & STORAGE**·HANDLING:**

Wash thoroughly after handling. Avoid contact with eyes, skin and clothing. Use only in a well-ventilated area. Use spark-proof tools and explosion-proof equipment. Do not breathe vapor or mist. Do not reuse container. Prevent build-up of electrostatic charges(e. g. grounding).

·STORAGE :

Keep container closed when not in use. Do not store in direct sunlight. Keep away from heat and flame. Separated from strong oxidants.

8.EXPOSURE CONTROL/PERSONAL PROTECTION**·CONTROL PARAMETERS:**

CHEMICAL NAME	CAS RN	ACGIH TWA (1995-1996)	OSHA(1996)	%100.0 (by wt.)
Bromopentafluorobenzene	344-04-7	Not established	Not established	More than 99%

·ENGINEERING MEASURES :

Facilities storing or utilizing this substance should be equipped with an eyewash facility and a safety shower. Use process enclosures, local exhaust ventilation, or other engineering controls.

·PERSONAL PROTECTIVE EQUIPMENT:

RESPIRATORY PROTECTION : Chemical cartridge respirator with an organic vapor cartridge. Positive-pressure self-contained breathing apparatus.

EYE PROTECTION : Wear safety glasses with side shields or goggles and a face shield.

HANDS,SKIN AND BODY PROTECTION:

Where contact is likely, wear chemical resistance gloves, a chemical suit, rubber boots.

9. PHYSICAL & CHEMICAL PROPERTIES

- **PHYSICAL STATE, FORM :** Liquid
- **APPEARANCE :** Colorless liquid
- **BOILING POINT :** 137°C²⁾
- **ODOUR :** Characterisitic
- **SOLUBILITY :** Insoluble in water . Soluble in toluene or acetone
- **DENSITY :** 1.981¹⁾
- **MELTING POINT :** -31°C¹⁾
- **VAPOUR PRESSURE :** Not applicable

10. PHYSICAL HAZARD(STABILITY & REACTIVITY)

- **FLASH POINT :** 87°C¹⁾ (Closed cup)
- **AUTOIGNITION TEMPERATURE :** Not available.
- **UPPER AND LOWER EXPLOSION LIMIT :** Not available.
- **FLAMMABILITY :** Combustible.
- **SPONTANEOUS COMBUSTIBILITY :** Not applicable.
- **REACTIVITY WITH WATER :** This substance is not reactive with water.
- **OXIDIZIBILITY :** This substance is not oxidant.
- **SELF-REACTIVITY :** Not applicable.
- **STABILITY & REACTIVITY:**
This substance is stable. Contact with bases may form fluorine salts. Contact of the salts with strong acids may liberate irritating, highly toxic and corrosive HF.
Incompatible with strong oxidants.
- **HAZARDOUS DECOMPOSITION PRODUCTS :**
When heated to decomposition it exits toxic gases. (Halogen gases, CO_x)

11. TOXICOLOGICAL INFORMATION

- **CORROSIVE AND IRRITANT PROPERTIES :**
 - Primary skin irritation test in rabbits : Slightly irritant.³⁾
 - Primary eye irritation test in rabbits : Slightly irritant.³⁾
- **ALLERGENIC AND SENSITIVE EFFECTS :** No information.
- **ACUTE TOXICITY :** oral-rat LD₅₀ : 2000mg/kg^{≤3)}
- **SUB-CHRONIC TOXICITY :** No information.
- **CHRONIC TOXICITY :** No information.
- **CARCINOGENIC EFFECTS :** Not established on IRCA, NTP, EU, OSHA.
- **MUTAGENIC EFFECTS :** This substance was negative in the Ames test.³⁾
This substance was positive in the chromosomal aberration test with S9 mix by metabolic activation method. D₂₀ values was 590 µg/ml.³⁾
- **SUB-CHRONIC TOXICITY :** A 28-day repeated-dose oral toxicity study in rats
; No-observed effects level was 10 mg/kg/day.
The 28-day reoeated-dose caused pathalogic change of liver and blood parameters of rats in 30-300 mg/kg/day.
In the 14-day recovery test, incisor of male and female rats in 300 mg/kg/day were given degeneration and irreguar alignment of the ameloblast at stage of maturation. Males in the 300 mg/kg/day were given a loss of incisor, swelling of the gingia and malocclusion in the same test.³⁾
- **EFFECTS ON THE REPRODUCTIVE :** No information.
- **TERATOGENIC EFFECTS :** No information.
- **HAZARDOUS DECOMPOSITION PRODUCTS :**
When heated to decomposition it emits toxic fumes. (Halogen gases, CO_x)

12. ECOLOGICAL INFORMATION

- **Biodegradability** : This substance is not biodegradable.³⁾
 - **Bioaccumulation** : This substance is not bioaccumulatable.³⁾
 - **Fish Toxicity** : $T_{1/2}m_{48}$ 20mg/l (Carp)³⁾
-

13. DISPOSAL CONSIDERATION

Burn in a chemical incinerator equipped with an afterburner and scrubber. Do not dump into sewers, on the ground or into any body or water. Do not pressurize, cut, weld, braze, solder, drill, grind, or expose such containers to heat, flame, sparks, static electricity or other sources of ignition. Empty drums should be completely drained, properly bunged and promptly returned to a drum reconditioner, or properly disposed of.

Adequate disposal are recommended, as toxic HF gas may liberate upon combustion.

14. TRANSPORT INFORMATION

Any transportation practice must be in compliance with laws and regulation in your country or region.

15. REGULATORY INFORMATION

- **US status**
 - TSCA Inventory : Listed
 - SARA TITLE 3 313 : Not listed

- **EU STATUS**
 - EINECS : Listed

· Regulatory information with regard to this substance in your country or region should be examined by your own responsibility.

16. OTHER INFORMATION

- **REFERENCES:** 1) Catalog Handbook of Fine Chemicals 1996-1997(Aldrich).
 - 2) These data are measured by
 - 3) Achieved by testing institute.

· To the best of our knowledge, the information contained herein is accurate. However, neither NIPPON SHOKUBAI CO.,LTD nor any of its subsidiaries assumes any liability whatsoever for the accuracy or completeness of the information contained herein. Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we can-not guarantee that these are the only hazards which exist.

TRIAGE of 8(e) Submissions

Date sent to triage: _____

NON-CAP

CAP

Submission number: 14021A

TSCA Inventory: Y N D

STUDY TYPE (circle appropriate):

Cheng-Chun Lee (E609C)

ATOX SBTOX SEN w/NEUR

Larry Newsome (E425)

ECO AQUATO

Katherine Anitole (E611G)

RTOX/DTOX

Daljit Sawhney (E611A)

CTOX STOX

Deborah Norris (E602)

NEUR

Jeff Beaubier (E608)

EPI

Ron Ward (E611F)

IMMUNO/ALLERG

David Lai (E611B)

CARC

Michael Cimino (E611D)

GTOX

Leonard Keifer (E611C)

META/PHARM

NOTES:

CREATES DATA

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CICCAT STRIAGE TRACKING DBASE ENTRY FORM

Submission # 00100-0197-140215

TYPE: INT. SUPP FLWP

SUBMITTER NAME: Confidential

INFORMATION REQUESTED: FLWP DATE:

0501 NO INFO REQUESTED:D

0502 INFO REQUESTED (TECH)

0503 INFO REQUESTED (VOL ACTIONS)

0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION:

0439 REFER TO CHEMICAL SCREENING

0576 CAP NOTICE

SUB. DATE: 9-10-97 ONS DATE: 9-22-97 CERAD DATE: 11-14-97

CHEMICAL NAME:

Bromo pentafluorobenzene

VOLUNTARY ACTIONS:

0401 TWO ACTION TO PURGE

0402 STUDIES PLANNED IN KOREA

0403 NOTIFICATION ON WORK IN KOREA

0404 LAMPJARDS CHAMPS

0405 PROCESSMANI INC, CHAMPS

0406 APP USE DISCONTINUED

0408 CONFIDENTIAL

0409 PRODUCTION DISCONTINUED

0410 APP USE DISCONTINUED

0411 CONFIDENTIAL

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"14021A"="M"="BROMOPENTAFLUOROBENZENE (CAS NO. 344-04-7):
CLASTOGENICITY WAS EVALUATED WITH CHINESES HAMSTER LUNG (CHL)
FIBROBLASTS BOTH WITH AND WITHOUT AROCLOR-INDUCED RAT LIVER S-9
METABOLIC ACTIVATION. CHROMOSOMAL ABERRATION TESTS WERE CARRIED
OUT USING: 1) 95, 190, AND 380 UG/ML OF THE TEST SUBSTANCE FOR 24 AND 48
HOUR TREATMENTS BY THE DIRECT METHOD; 2) 137.5, 275, AND 550 UG/ML IN
THE GROUP WITHOUT S9 MIX; AND 3) 400, 500, AND 600 UG/ML IN THE GROUP
WITH S9 MIX. THE INCIDENCE OF STRUCTURAL CHROMOSOMAL ABERRATIONS
IN THE GROUP WITH ACTIVATION WAS INCREASED AT 400-500 UG/ML, BUT
DECREASED TO 4.5% AT 600 UG/ML. A REEXAMINATION WITH THE SAME DOSES
SHOWED THAT THE TEST SUBSTANCE INDUCED STRUCTURAL CHROMOSOMAL
ABERRATIONS DOSE-DEPENDENTLY WITHIN THE DOSE RANGE OF 400 TO 600
UG/ML IN THE GROUP WITH S9. IT WAS CONCLUDED THAT BPFB INDUCED
CHROMOSOMAL ABERRATIONS IN CHL CELLS UNDER THE TEST CONDITIONS.

"14021A" = "L" = "BROMOPENTAFLUOROBENZENE (CAS NO. 344-04-7): A 28-DAY REPEATED DOSE ORAL TOXICITY STUDY WAS CONDUCTED. MALE AND FEMALE CRJ: CD (SD) RATS (6/SEX/GROUP) WERE EXPOSED VIA GAVAGE TO EITHER 0, 10, 30, 100, OR 300 MG/KG/DAY OF THE TEST SUBSTANCE DISSOLVED IN OLIVE OIL. ADDITIONAL ANIMALS AT 0, 100, AND 300 MG/KG WERE MAINTAINED FOR A TWO WEEK RECOVERY PERIOD. NO DEATHS OCCURRED AND NO CHANGES IN BODY WEIGHT OR FOOD CONSUMPTION WERE OBSERVED. CLINICAL SIGNS INCLUDED DECREASED SPONTANEOUS LOCOMOTION AND SALIVATION IN BOTH SEXES AT 300 MG/KG, AND STAINING OF THE LOWER ABDOMEN AND AROUND THE ANUS, AND MOIST ABDOMINAL HAIR IN FEMALES AT 300 MG/KG. EXAMINATION OF HEMATOLOGICAL PARAMETERS SHOWED DECREASED PLATELET COUNTS AND AN INCREASE IN MEAN CORPUSCULAR HEMOGLOBIN IN HIGH DOSE MALES. EXAMINATION OF BLOOD CHEMISTRY REVEALED: INCREASED INORGANIC PHOSPHORUS IN MALES AT 30 MG/KG AND HIGHER; INCREASED TOTAL BILIRUBIN IN FEMALES AT 100 AND 300; INCREASES IN TOTAL CHOLESTEROL LEVELS IN THE 300 MG/KG MALES AND FEMALES; INCREASED GPT ACTIVITY AND ALKALINE PHOSPHATASE LEVELS IN MALES AT 300 MG/KG; AND INCREASED GOT ACTIVITY, INCREASED TRIGLYCERIDE AND INORGANIC PHOSPHORUS LEVELS, AND DECREASED CHOLINESTERASE LEVEL IN HIGH DOSE FEMALES. AT THE END OF THE RECOVERY PERIOD, INCREASED GTP ACTIVITY WAS STILL SEEN IN HIGH DOSE MALES. INCREASED URINE VOLUMES WERE NOTED IN BOTH SEXES AT THE HIGH DOSE. ALTERED ORGAN WEIGHTS INCLUDED INCREASED LIVER WEIGHTS IN BOTH SEXES AT 30 MG/KG AND HIGHER, DECREASED SPLEEN WEIGHTS IN MALES AT 300 MG/KG, AND INCREASED KIDNEY WEIGHT IN FEMALES AT 300 MG/KG. NECROSPY REVEALED ENLARGEMENT OF THE LIVER IN BOTH SEXES AT 300 MG/KG. HISTOPATHOLOGICAL EXAMINATION REVEALED SWELLING AND GROUND GLASS APPEARANCE OF THE HEPATOCYTES IN 300 MG/KG FEMALES. PROMINENT NUCLEOLI OF THE HEPATOCYTES WAS NOTED IN HIGH DOSE MALES. RECOVERY ANIMALS HAD INCREASED GPT ACTIVITY (300 MG/KG

MALES) AND STAINING OF THE ANUS (300 MG/KG FEMALES). WHITISH REGIONS OF THE INCISOR IN BOTH SEXES (300 MG/KG), AND LOSS OF INCISORS, SWELLING OF THE GINGIVA, AND MALOCCLUSION (300 MG/KG MALES) WERE ALSO NOTED. HISTOPATHOLOGICAL EXAMINATION OF THE INCISORS REVEALED DEGENERATION AND IRREGULAR ALIGNMENT OF THE AMELOBLASTS AT THE STAGE OF MATURATION IN BOTH SEXES EXPOSED TO 300 MG/KG. THE NOEL FOR THIS STUDY WAS CONSIDERED TO BE 10 MG/KG/DAY.